



**INTERACTION EFFECTS BETWEEN THE
PEACH POTATO APHID *MYZUS PERSICAE*
AND SECONDARY PLANT METABOLITES
OCCURRING IN POTATO *SOLANUM*
TUBEROSUM L.**

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Abstract

Peach potato aphids *Myzus persicae* (Sulzer) (Homoptera: Aphididae) were reared on artificial diets containing the steroidal glycoalkaloids (GAs) α -solanine and α -chaconine in concentrations lower or similar to those observed in potato leaves. The parameters used to measure the aphid performance were the total individual adult cumulative fecundity, diet uptake and mortality the mean relative growth rate (MRGR) and the intrinsic rate of natural increase (IRI). The adults proved to be susceptible to high concentrations (80-160 mg GA /100ml of diet) showing reduced fecundity, diet uptake and increased mortality in comparison to controls. Ingestion of these artificial diets by nymphs delayed maturity and decreased the intrinsic rate of natural increase. GAs in low concentrations marginally stimulated the reproductive performance and diet acceptability of this aphid.

The effects of nitrogen fertilisation and pesticide (Aldicarb) application on the foliar total and individual GA production of two potato cultivars King Edward and Maris Piper were examined using a semi-hydroponic culture system. Nitrogen deficient potato plants produced elevated concentrations of GAs. The two cultivars reacted in a different way on pesticide application. Cv. King Edward produced elevated amounts of GAs in the low pesticide treatment when no differences were observed between pesticide treatments on cv. Maris Piper.

Glycoalkaloid production of potato plants subjected to stress induced by peach potato aphid *M. persicae* infestation was investigated in a glasshouse, a plant growth room, and an open-air field experiment. In both glasshouse and plant growth room experiments aphid infested potato plants produced reduced amounts of total and individual GAs compared to these produced by non infested plants. This reduction was attributed to the sugar deficiency induced to the plants due to the dense aphid colonisation. However results from the field experiment showed a temporal increase of the glycoalkaloids produced by the potato cv. King Edward plants subjected to aphid infestation. It is concluded that aphid infestation as a stress inducing factor possibly affects that part of secondary metabolism responsible for the glycoalkaloid production in potato.

Dedication

This work is dedicated to my parents who are always encouraging me towards life.

Που 'ναι η αγάπη πού κόβει τον καιρό μονοκόμματα στα δύο καί τόν αποσβολώνει;

Where is love that with one stroke cuts time in two and stuns it?

*-From "The mood of a Day"
by George Seferis*

Declaration

I, the undersigned, hereby declare that this thesis has been composed entirely by myself and that all the work carried out herein is my own, except where specifically stated.

Demetrios A. Fragoyiannis
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Table of contents

CHAPTER 1:General introduction, background and rationale	1
1.1 Plants and environmental stress	2
1.2 Secondary metabolites and their role in plants	3
1.3 Plant stress and allelochemicals	5
1.4 Secondary plant metabolites and their role in aphid feeding behaviour	6
1.4.1 Indole alkaloids	8
1.4.2 Quinolizidine alkaloids	9
1.4.3 Hydroxamic acids	10
1.4.4 Glucosinolates	11
1.4.5 Flavonoid and phenolic compounds	13
1.4.6 Other compounds	14
1.5 Glycoalkaloids	15
1.6 Distribution of glycoalkaloids in the potato plant	17
1.7 Biosynthesis of glycoalkaloids	18
1.8 Glycoalkaloid synthesis as affected by different factors	20
1.8.1 Photoinduction	20
1.8.2 Cultivar	21
1.8.3 Location, climate and environment	22
1.8.4 Physiological stress	25
1.9 Pharmacology and toxicology	26
1.10 Effects of <i>Solanum</i> glycoalkaloids on the major potato pests	27
1.11 Potato glycoalkaloids as resistance factors against the peach potato aphid <i>Myzus persicae</i> (Sulzer)	32
1.12 Objectives of the study	34
CHAPTER 2:Effects of potato glycoalkaloids on life parameters of the aphid <i>Myzus persicae</i>	37
2.1 Introduction	38
2.2 Materials and Methods	38

2.2.1	Host plant culture for the rearing of the aphid colony	39
2.2.2	Rearing procedure	39
2.2.3	Artificial diet formulation and method of use	40
2.2.4	Osmolality measurements	42
2.2.5	Procedures and assessment of aphid performance	43
2.3	Results	45
2.3.1	Preliminary experiment (pH exp.)	45
2.3.2	Adult experiment	48
2.3.3	Nymph experiment	53
2.4	Discussion	60

CHAPTER 3: Effects of nitrogen fertilisation and pesticide application, on the foliar glycoalkaloid production of potato

3.1	Introduction	66
3.2	Materials and Methods	68
3.2.1	Growing media	68
3.2.2	Plant material	69
3.2.3	Nutrient solution and pesticide application	69
3.2.4	Sample preparation	72
3.2.5	Sample extraction and purification	72
3.2.6	Apparatus and Reagents	73
3.2.7	Calculation for conversion of area under peak to glycoalkaloid concentration mg/100g fresh weight (FW) of leaf tissue	74
3.3	Results	75

Experiment 1

3.3.1	Effects of nitrogen fertilisation on green matter, dry matter, total and individual glycoalkaloids	75
3.3.2	Effects of pesticide application on green matter, dry matter, total and individual glycoalkaloids	78

Experiment 2

3.3.3	Effects of nitrogen fertilisation on green and dry matter	82
3.3.4	Effects of nitrogen fertilisation on total glycoalkaloids	85
3.3.5	Effects of pesticide application on green and dry matter	88
3.3.6	Effects of pesticide application on total glycoalkaloids	89
3.3.7	Effects of nitrogen fertilisation and pesticide application on individual glycoalkaloids	96

CHAPTER 4: Effects of aphid infestation on the foliar glycoalkaloid production of potato, grown in protected environment 106

4.1	Introduction	107
4.2	Materials and Methods	109
4.2.1	Growing media	109
4.2.2	Plant material	109
4.2.3	Nutrient solution and aphid infestation	109
4.2.4	Assessment of aphid performance	112
4.3	Results	114

Experiment 1

4.3.1	Effects of nitrogen fertilisation and aphid infestation on green and dry matter	114
4.3.2	Effects of nitrogen fertilisation and aphid infestation on total glycoalkaloid production	118
4.3.3	Effects of nitrogen fertilisation and aphid infestation on individual glycoalkaloid production	125
4.3.4	Aphid performance	129

Experiment 2

4.3.5	Effects of nitrogen fertilisation, aphid infestation and cultivar on green and dry matter	130
4.3.6	Effects of nitrogen fertilisation and aphid infestation on total glycoalkaloid production	135
4.3.7	Effects of nitrogen fertilisation, aphid infestation and cultivar on individual glycoalkaloid production	146
4.3.8	Aphid performance	150
4.3.9	Stress and after stress effects induced by aphid infestation in both experiments	152
4.4	Discussion	156

CHAPTER 5: Effects of aphid infestation on the foliar glycoalkaloid production of potato, grown in the field 161

5.1	Introduction	162
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5.2	Materials and Methods	163
5.2.1	Growing media	163
5.2.2	Plant material	164
5.2.3	Assessment of aphid performance	166
5.3	Results	168
5.3.1	Effects of fertilisation, cultivar and aphid infestation on green and dry matter	168
5.3.2.	Effects of fertilisation and aphid infestation on total glycoalkaloid production	172
5.3.3	Effects of fertilisation and cultivar on individual glycoalkaloid production	184
5.3.4	Aphid performance	184
5.4	Discussion	186
CHAPTER 6: General discussion, conclusions and future prospects		190
6.1	General Discussion	191
6.2	Summary of major findings	203
6.3	Recommendations for future work	206
Bibliography		208
Appendices		229

List of Tables

Table

1.1.	Occurrence and composition of α -, β - and γ - forms of solanine and chaconine found in <i>Solanum tuberosum</i> L.	16
1.2.	Distribution of total glycoalkaloids in the potato plant and various tuber tissues (After Wood and Young, 1974)	17
2.1.	Composition of artificial diet	41
2.2.	Fecundity of <i>Myzus persicae</i> adults fed on artificial diets containing different concentrations of glycoalkaloids	49
2.3.	Life parameters (mean relative growth rate, ecdysis rate and intrinsic rate of natural increase) of nymphs reared on artificial diets containing different concentrations of glycoalkaloids.	55
2.4.	Mortality, of <i>Myzus persicae</i> nymphs reared on artificial diets containing different concentrations of glycoalkaloids.	59
3.1.	Recipes for stock solutions (g/l) diluted approximately 100 times to prepare the feeds applied to potato plants	70
3.2.	Composition of diluted feeds (mg/l) applied to potato plants after approximately 100x dilution	70
3.3a.	Effects of nitrogen (N) fertilisation on green matter, dry matter and total foliar fresh and dry weight glycoalkaloid production of potato cv. King Edward	76
3.3b.	Effects of nitrogen (N) fertilisation on individual foliar glycoalkaloid production of potato cv. King Edward	78
3.4a.	Effects of pesticide application on green matter, dry matter, and total foliar fresh and dry weight glycoalkaloid production of potato cv. King Edward	79
3.4b.	Effects of pesticide application on individual foliar glycoalkaloid production of potato cv. King Edward	81
3.5a.	Effects of nitrogen (N) fertilisation on green and dry matter production of potato cvs. King Edward and Maris Piper	83
3.5b.	Interaction effects of nitrogen (N) fertilisation x cultivar on green and dry matter production of potato cvs. King Edward and Maris Piper	83

3.5c.	Effects of cultivar on green and dry matter production of potato cvs. King Edward and Maris Piper	84
3.6a.	Effects of nitrogen (N) fertilisation on fresh weight, dry weight and total foliar glycoalkaloid production of potato cvs. King Edward and Maris Piper	85
3.6b.	Interaction effects of nitrogen (N) fertilisation x cultivar on fresh weight, dry weight and total foliar glycoalkaloid production of potato cvs. King Edward and Maris Piper	87
3.6c.	Effects of cultivar on fresh weight, dry weight and total foliar glycoalkaloid production of potato cvs. King Edward and Maris Piper	88
3.7a.	Effects of pesticide application on green and dry matter production of potato cvs. King Edward and Maris Piper	88
3.7b.	Interaction effects of pesticide application x cultivar on green and dry matter production of potato cvs. King Edward and Maris Piper	89
3.8a.	Effects of pesticide application on fresh weight, dry weight and total glycoalkaloid production of potato cvs. King Edward and Maris Piper	90
3.8b.	Interaction effects of pesticide application x cultivar on fresh weight, dry weight and total glycoalkaloid production of potato cvs. King Edward and Maris Piper	90
3.9a.	Effects of nitrogen (N) fertilisation on the individual glycoalkaloid production of potato cvs. King Edward and Maris Piper	96
3.9b.	Interaction effects of nitrogen (N) fertilisation x cultivar on the individual glycoalkaloid production of potato cvs. King Edward and Maris Piper	97
3.9c.	Effects of cultivar on the individual glycoalkaloid production of potato cvs. King Edward and Maris Piper	97
3.10a.	Effects of pesticide application on the individual glycoalkaloid production of potato cvs. King Edward and Maris Piper	98
3.10b.	Interaction effects of pesticide application x cultivar on the individual glycoalkaloid production of potato cvs. King Edward and Maris Piper	98
4.1a.	Effects of nitrogen (N) fertilisation on the green (FW) and dry matter (DW/FW) produced by potato plants cv. King Edward	115

- 4.1b.** Effects of aphid infestation on the green (FW) and dry matter (DW/FW) produced by aphid infested (G2, G4) and non-infested (G1, G3, G5) potato plants cv. King Edward 116
- 4.1c.** Interaction effects of nitrogen (N) fertilisation x aphid infestation on the green (FW) and dry matter (DW/FW) produced by aphid infested (G2, G4) and non-infested (G1, G3, G5) potato plants cv. King Edward 117
- 4.2a.** Effects of nitrogen (N) fertilisation on individual foliar glycoalkaloids produced by potato plants cv. King Edward 125
- 4.2b.** Effects of aphid infestation on individual foliar glycoalkaloids produced by five different groups of aphid infested (G2, G4) and non-infested (G1, G3, G5) potato plants cv. King Edward 127
- 4.2c.** Interaction effects of nitrogen (N) fertilisation x aphid infestation on individual foliar glycoalkaloids produced by five different groups of aphid infested (G2, G4) and non-infested (G1, G3, G5) potato plants cv. King Edward 128
- 4.3.** Fecundity of *Myzus persicae* adults fed on potato plants cv. King Edward grown in four different nutrient solutions 130
- 4.4a.** Interaction effects of nitrogen (N) fertilisation x cultivar on the green (FW) and dry matter (DW/FW) produced by potato plants cvs. King Edward and Maris Piper 130
- 4.4b.** Interaction effects of nitrogen (N) fertilisation x aphid infestation on the green (FW) and dry matter (DW/FW) produced by aphid infested (G2, G4) and non-infested (G1, G3, G5) potato plants cvs. King Edward and Maris Piper 132
- 4.4c.** Interaction effects of cultivar x aphid infestation on the green (FW) and dry matter (DW/FW) produced by aphid infested (G2, G4) and non-infested (G1, G3, G5) potato plants cvs. King Edward and Maris Piper 133
- 4.4d.** Interaction effects of nitrogen (N) fertilisation x aphid infestation x cultivar on the green (FW) and dry matter (DW/FW) produced by aphid infested (G2, G4) and non-infested (G1, G3, G5) potato plants cvs. King Edward and Maris Piper 134
- 4.5a.** Interaction effects of nitrogen (N) fertilisation x cultivar on individual foliar glycoalkaloids produced by potato plants cvs. King Edward and Maris Piper 146

4.5b.	Effects of aphid infestation on individual foliar glycoalkaloids produced by five different groups of aphid infested (G2, G4) and non-infested (G1, G3, G5) potato plants cvs. King Edward and Maris Piper	148
4.5c.	Interaction effects of nitrogen (N) fertilisation x aphid infestation x cultivar on individual foliar glycoalkaloids produced by five different groups of aphid infested (G2, G4) and non-infested (G1, G3, G5) potato plants cvs. King Edward and Maris Piper	149
4.6a.	Effects of nitrogen fertilisation on the fecundity of <i>Myzus persicae</i> adults fed on potato plants cvs. King Edward and Maris Piper grown in two different nutrient solutions	150
4.6b.	Interaction effects of nitrogen fertilisation x cultivar on the fecundity of <i>Myzus persicae</i> adults fed on potato plants cvs. King Edward and Maris Piper grown in two different nutrient solutions	151
4.6c.	Effects of cultivar on the fecundity of <i>Myzus persicae</i> adults fed on potato plants cvs. King Edward and Maris Piper grown in two different nutrient solutions	151
4.7a.	Stress and after stress effects of aphid infestation on the fresh weight total foliar glycoalkaloid production of potato cv. King Edward (Exp. 1)	155
4.7b.	Stress and after stress effects of aphid infestation on the fresh weight total foliar glycoalkaloid production of potato cvs. King Edward and Maris Piper (Exp. 2)	155
5.1.	Soil Analysis Report	164
5.2a.	Interaction effects of fertilisation x cultivar on the green (FW) and dry matter (DW/FW) produced by potato plants cvs. King Edward and Maris Piper	168
5.2b.	Interaction effects of fertilisation x aphid infestation on the green (FW) and dry matter (DW/FW) produced by aphid infested (G2, G4) and non-infested (G1, G3, G5) potato plants cvs. King Edward and Maris Piper	170
5.2c.	Interaction effects of fertilisation x aphid infestation x cultivar on the green (FW) and dry matter (DW/FW) produced by aphid infested (G2, G4) and non-infested (G1, G3, G5) potato plants cvs. King Edward and Maris Piper	171
5.3.	Interaction effects of fertilisation x cultivar on individual foliar glycoalkaloids produced by potato plants cvs. King Edward and Maris Piper	184

- 5.4a.** Effects of fertilisation on the fecundity of *Myzus persicae* adults fed on potato plants cvs. King Edward and Maris Piper grown with manure and fertiliser 185
- 5.4b.** Interaction effects of fertilisation x cultivar on the fecundity of *Myzus persicae* adults fed on potato plants cvs. King Edward and Maris Piper grown with manure and fertiliser 185

List of Figures

Figure

- 1.1. The biosynthetic path of glycoalkaloid synthesis after Jadhav and Salunkhe, (1975). 19
- 2.1. Mean cumulative fecundity of *Myzus persicae* adults fed on non-acidic (pH=7.0), medium acidic (pH=6.0) and highly acidic (pH=4.6) artificial diets for the first five days of their reproductive life. 46
- 2.2. Mean cumulative fecundity of *Myzus persicae* adults fed on non-acidic (pH=7.0), medium acidic (pH=6.0) and highly acidic (pH=4.6) artificial diets at eight different time intervals for the entire reproductive period (from 3 to 24 days). 47
- 2.3. Mortality of *Myzus persicae* adults fed on artificial diets of different acidity (pH= 4.6-7.0), at five different time intervals across lifespan (from 6 to 30 days). 47
- 2.4. Diet uptake by *Myzus persicae* adults and offspring during 48 hours of feeding on artificial diets of different acidity (pH= 4.6-7.0). 48
- 2.5. Mean cumulative fecundity of *Myzus persicae* adults fed on artificial diets containing different glycoalkaloid levels (T_0 - T_{160} mg/100ml diet), for the first five days of reproductive life. 50
- 2.6. Mean cumulative fecundity of *Myzus persicae* adults fed on artificial diets containing different glycoalkaloid levels (T_0 - T_{160} mg/100ml diet), at six different time intervals for the entire reproductive period (from 4 to 24 days). 51
- 2.7. Mortality of *Myzus persicae* adults fed on artificial diets containing different glycoalkaloid levels (T_0 - T_{160} mg/100ml diet), at seven different time intervals across lifespan (from 5 to 35 days) 52
- 2.8. Diet uptake by *Myzus persicae* adults and offspring during 48 hours of feeding on artificial diets containing different glycoalkaloid levels (T_0 - T_{160} mg/100ml diet). 53
- 2.9. Weight increase of *Myzus persicae* nymphs reared on artificial diets containing different glycoalkaloid levels (T_0 - T_{160} mg/100ml diet), at 12, 72, 120 and 156 hours of feeding. 54
- 2.10. Mean relative growth rates of *Myzus persicae* nymphs reared on artificial diets containing different glycoalkaloid levels (T_0 - T_{160} mg/100ml diet) at three different time intervals (72, 120 and 156 hours of feeding). 56

- 2.11. Ecdyses of *Myzus persicae* nymphs reared on artificial diets containing different glycoalkaloid levels (T_0 - T_{160} mg/100ml diet) in 72, 120 and 156 hours of feeding. 57
- 2.12. Reproductive performance of adults reared on artificial diets containing different glycoalkaloid levels (T_0 - T_{160} mg/100ml diet) and weight of new born *Myzus persicae* nymphs. 59
- 3.1. Total content (mg/100g) and total per plant (mg) foliar fresh weight (FW) glycoalkaloid production for four nitrogen (N_1 , N_2 , N_3 and N_4) fertilisation levels applied (Exp. 1). 77
- 3.2. Total content (mg/100g) and total per plant (mg) foliar dry weight (DW) glycoalkaloid production for four nitrogen (N_1 , N_2 , N_3 and N_4) fertilisation levels applied (Exp. 1). 77
- 3.3. Total content (mg/100g) and total per plant (mg) foliar fresh weight (FW) glycoalkaloid production for three pesticide (P_0 , P_1 and P_2) levels applied. 79
- 3.4. Total content (mg/100g) and total per plant (mg) foliar dry weight (DW) glycoalkaloid production for three pesticide (P_0 , P_1 and P_2) levels applied. 80
- 3.5. Total foliar glycoalkaloid production affected by nitrogen (N) fertilisation for three pesticide levels (P_0 , P_1 and P_2) expressed in mg of glycoalkaloids per 100g of fresh weight (FW). 81
- 3.6. Total foliar glycoalkaloid production affected by nitrogen (N) fertilisation for three pesticide levels (P_0 , P_1 and P_2) expressed in mg of glycoalkaloids per 100g of dry weight (DW). 82
- 3.7. Total content (mg/100g) and total per plant (mg) foliar fresh weight (FW) glycoalkaloid production for four nitrogen (N_1 , N_2 , N_3 and N_4) fertilisation levels applied (KE+MP means, Exp. 2). 86
- 3.8. Total content (mg/100g) and total per plant (mg) foliar dry weight (DW) glycoalkaloid production for four nitrogen (N_1 , N_2 , N_3 and N_4) fertilisation levels applied (KE+MP means, Exp. 2). 86
- 3.9. Total content (mg/100g) and total per plant (mg) foliar fresh weight (FW) glycoalkaloid production for three pesticide (P_0 , P_1 and P_2) levels applied (KE+MP means, Exp.2). 91
- 3.10. Total content (mg/100g) and total per plant (mg) foliar dry weight (DW) glycoalkaloid production for three pesticide (P_0 , P_1 and P_2) levels applied (KE+MP means, Exp.2). 91

- 3.11. Total foliar glycoalkaloid production of both potato cvs. (KE+MP) as affected by nitrogen (N) fertilisation for three pesticide levels (P_0 , P_1 and P_2) expressed in mg of glycoalkaloids per 100g of fresh weight (FW). 92
- 3.12. Total foliar glycoalkaloid production of both potato cvs. (KE+MP) as affected by nitrogen (N) fertilisation for three pesticide levels (P_0 , P_1 and P_2) expressed in mg of glycoalkaloids per 100g of dry weight (DW). 92
- 3.13. Total foliar glycoalkaloid production of potato cv. King Edward as affected by nitrogen (N) fertilisation for three pesticide levels (P_0 , P_1 and P_2) expressed in mg of glycoalkaloids per 100g of fresh weight (FW). 93
- 3.14. Total foliar glycoalkaloid production of potato cv. Maris Piper as affected by nitrogen (N) fertilisation for three pesticide levels (P_0 , P_1 and P_2) expressed in mg of glycoalkaloids per 100g of fresh weight (FW). 95
- 4.1. Schematic representation of the *Myzus persicae* infestation groups (G1, G2, G3, G4 and G5) applied to the potato plants plotted against times of harvesting; +A: aphid-infested period, -A: aphid-free period. 111
- 4.1a & 4.1b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids produced by aphid-infested (I.-G2 & G4) and non-infested (N.I.-G1, G3 & G5) plants harvested at three different time intervals. 118
- 4.2a & 4.2b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids produced by aphid-infested (G2) and non-infested (G3) plants for four nitrogen (N_1 , N_2 , N_3 and N_4) fertilisation levels (Exp. 1). 120
- 4.3a & 4.3b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids produced by aphid-infested (G4) and non-infested (G5) plants for four nitrogen (N_1 , N_2 , N_3 and N_4) fertilisation levels (Exp. 1). 121
- 4.4a & 4.4b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids produced by non-infested plants grown in four different nutrient solutions (N_1 , N_2 , N_3 and N_4) and harvested at three different developmental stages (G1, G3 and G5) (Exp. 1). 122
- 4.5a & 4.5b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids produced by aphid-infested plants grown in four different nutrient solutions (N_1 , N_2 , N_3 and N_4) and harvested at two different developmental stages (G2 and G4) (Exp. 1). 124

- 4.6. Mean cumulative fecundity of *Myzus persicae* adults infesting potato plants grown in four different nitrogen solutions. 129
- 4.7a & 4.7b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids produced by aphid-infested (I. - G2 & G4) and non-infested (N.I. - G1, G3 & G5) potato plants cv. King Edward harvested at three different time intervals. 135
- 4.8a & 4.8b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids produced by aphid-infested (G2) and non-infested (G3) potato plants cv. King Edward for two nitrogen (N_2 and N_4) fertilisation levels (Exp. 2). 136
- 4.9a & 4.9b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids produced by aphid-infested (G4) and non-infested (G5) potato plants cv. King Edward for two nitrogen (N_2 and N_4) fertilisation levels (Exp. 2). 138
- 4.10a & 4.10b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids produced by non-infested potato plants cv. King Edward, grown in two different nutrient solutions (N_2 and N_4) and harvested at three different developmental stages (G1, G3 and G5) (Exp. 2). 139
- 4.11a & 4.11b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids produced by aphid-infested potato plants cv. King Edward, grown in two different nutrient solutions (N_2 and N_4) and harvested at two different developmental stages (G2 and G4) (Exp. 2). 140
- 4.12a & 4.12b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids produced by aphid-infested (G2 & G4) and non-infested (G1, G3 & G5) potato plants cv. Maris Piper harvested at three different time intervals. 141
- 4.13a & 4.13b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids produced by aphid-infested (G2) and non-infested (G3) potato plants cv. Maris Piper for two nitrogen (N_2 and N_4) fertilisation levels (Exp. 2). 142
- 4.14a & 4.14b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids produced by aphid-infested (G4) and non-infested (G5) potato plants cv. Maris Piper for two nitrogen (N_2 and N_4) fertilisation levels (Exp. 2). 143

- 4.15a & 4.15b.** Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids produced by non-infested potato plants cv. Maris Piper, grown in two different nutrient solutions (N₂ and N₄) and harvested at three different developmental stages (G1, G3 and G5) (Exp. 2). 144
- 4.16a & 4.16b.** Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids produced by aphid-infested potato plants cv. Maris Piper grown in two different nutrient solutions (N₂ and N₄) and harvested at two different developmental stages (G2 and G4) (Exp. 2). 145
- 4.17.** Total content (mg/100g) of foliar fresh (FW) weight glycoalkaloids produced by aphid-infested (I.) and non-infested (N.I.) plants harvested at three different time intervals examined separately for each nitrogen level applied (Exp. 1). 153
- 4.18.** Total content (mg/100g) of foliar fresh (FW) weight glycoalkaloids produced by aphid-infested (I.) and non-infested (N.I.) plants harvested at three different time intervals examined separately for each nitrogen level applied in potato cvs. King Edward and Maris Piper (Exp. 2). 154
- 5.1.** Schematic representation of the *Myzus persicae* infestation groups (G1, G2, G3, G4 and G5) applied to the potato plants plotted against times of harvesting; +A: aphid-infested period, -A: aphid-free period. 165
- 5.1a & 5.1b.** Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids produced by manure grown, aphid-infested (I. - G2 & G4) and non-infested (N.I. - G1, G3 & G5) potato plants cv. King Edward, harvested at three different time intervals. 172
- 5.2a & 5.2b.** Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids produced by fertiliser grown, aphid-infested (I. - G2 & G4) and non-infested (N.I. - G1, G3 & G5) potato plants cv. King Edward, harvested at three different time intervals. 174
- 5.3a & 5.3b.** Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids produced by aphid-infested (G2) and non-infested (G3) potato plants cv. King Edward grown under manure and nitrogen fertilisation. 175
- 5.4a & 5.4b.** Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids produced by aphid-infested (G4) and non-infested (G5) potato plants cv. King Edward grown under manure and nitrogen fertilisation. 176

- 5.5a & 5.5b.** Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids produced by non-infested potato plants cv. King Edward, grown under manure and nitrogen fertilisation and harvested at three different developmental stages (G1, G3 and G5). 178
- 5.6a & 5.6b.** Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids produced by manure grown, aphid-infested (I. - G2 & G4) and non-infested (N.I. - G1, G3 & G5) potato plants cv. Maris Piper, harvested at three different time intervals. 179
- 5.7a & 5.7b.** Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids produced by fertiliser grown, aphid-infested (I. - G2 & G4) and non-infested (N.I. - G1, G3 & G5) potato plants cv. Maris Piper, harvested at three different time intervals. 180
- 5.8a & 5.8b.** Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids produced by aphid-infested (G2) and non-infested (G3) potato plants cv. Maris Piper grown under manure and nitrogen fertilisation. 181
- 5.9a & 5.9b.** Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids produced by aphid-infested (G4) and non-infested (G5) potato plants cv. Maris Piper grown under manure and nitrogen fertilisation. 182
- 5.10a & 5.10b.** Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids produced by non-infested potato plants cv. Maris Piper, grown under manure and nitrogen fertilisation and harvested at three different developmental stages (G1, G3 and G5). 183

Chapter 1

General introduction, background and rationale

1.1 Plants and environmental stress

Plants are autotrophic organisms, which through photosynthesis can turn inorganic raw materials into organic (sugars, vitamins, amino acids etc.). All plants are dependent on their environment for growth and development. Environment includes all of the factors and forces prevailing internally and externally on, around, and in the plant (Treshow, 1970). Agricultural productivity is determined by a combination of interactions of plants with their abiotic and biotic environments. Crop production, which is the management of useful plants, can be regarded as the basis of our civilisation (Forbes & Watson, 1992). Throughout the centuries people either have been changing or selecting the crop plants that survive in hostile environments, or have been optimising the environment to suit the crop plants (Janick, 1974). Changing of crop plants has been achieved either through genetic selection and plant breeding, or by applying chemicals which alter the rate or the pattern of plant growth (Scott, 1998; Leopold, 1975). Environment optimisation is achieved by providing near to optimum amounts of water (e.g. irrigation and drainage), inorganic nutrients (e.g. fertilisation), and climate conditions (e.g. glasshouses). Climate is a major abiotic factor determining what crops will be grown and what production will be (Heinrichs, 1988). The Irish potato famine in the 1840s is a paragon of a climate related food disaster. Cool damp weather contributed to the blight that dramatically reduced potato production (Schneider & Bach, 1981). The potato famine reduced the Irish population by 50% due to death and migration.

Biologists have used the term “stress” for any environmental factor potentially unfavourable to living organisms (Levitt, 1980). According to Jaffe & Telewski (1984), “stress is an environmentally induced change in a plant which is potentially injurious”. Grime (1981), has defined plant stress as “external constraints which limit the rate of dry matter production”. Heinrichs (1988), considered stress as “any abiotic or biotic factor of the environment that affects plant physiology, chemistry, growth, and/or development in such a way that plants perform below the average for a region”. Agricultural ecosystems are subject to pest and pathogen infestations

because they provide ideal habitats for certain species of insect herbivores or plant pathogens. Crop cultivars that have been bred for high yields on soils treated with manure and fertilisers are grown in these ecosystems throughout the year. But when these cultivars are grown under suboptimal conditions they may become highly susceptible to insect infestations (Dale, 1988). Pest and pathogen infestations can cause severe losses by reducing yields and quality of the crops and by increasing production costs. Herbivorous insects in particular, can damage plants directly by feeding on all plant parts in all developmental stages and indirectly by being vectors of plant pathogens.

Herbivorous insects are considered biotic agents that can cause chemical stress to plants directly or indirectly through their feeding activity. The way that crops are protected from pests and pathogens is one of the factors that determine the type of farming which is applied to a crop. In organic farming, plant protection from pests and pathogens is achieved either by the application of pesticides that can be found in nature (mainly as plant extracts), or by increasing the populations of natural enemies and predators, or by a combination of the two. In addition utilisation of intercropping techniques of cultivation contributes to pest management. In conventional farming, plant protection is dependent exclusively on synthetic products that have been chemically prepared and are usually applied in a monoculture cultivating system.

1.2 Secondary metabolites and their role in plants

Through evolution, plants developed mechanisms of defence against herbivorous insects. The nature of these mechanisms is related to the morphological and anatomical characteristics of the plant (tough epidermis, cuticular deposits, etc.). Apart from creating these physical or mechanical barriers, plants in addition defend themselves against insects employing chemical protection and producing secondary plant compounds or metabolites. Secondary plant metabolites were considered to be metabolic waste products with no definite function (Schoonhoven, 1972). In

particular Schoonhoven (1972), has defined secondary plant compounds as “those compounds that are not universally found in higher plants but are restricted to certain plant taxa or occur in certain plant taxa at much higher concentrations than in others and are of no nutritional significance to insects”.

Almost all plants contain some chemical substances to defend against insect herbivory. These are considered to be either “quantitative” defences (digestibility-reducing substances) which are common in e.g. long lived trees, or “qualitative” chemical defences (toxins) which are present in early successional herbaceous plants (Rhoades & Cates, 1976; Futuyma, 1976; Feeny, 1976). The first category of quantitative defences comprises primarily carbon-based compounds like phenolics, tannins, and various terpenoid compounds (Harborne, 1977). The second category of qualitative defences comprises nitrogen-based compounds and among others includes the alkaloids, the non-protein amino acids, cyanogenic glycosides and glucosinolates. The qualitative defences are usually associated with nitrogen-rich plant tissue (newly developing tissue) and reproductive organs like seeds (Harborne, 1993). Secondary plant compounds which are produced by an organism and affect other organisms are referred to as allelochemicals (Kogan, 1986). Allelochemicals are considered to be allomones if their activity benefits the producing organism and kairomones if their activity favours the receiving organism (Heinrichs, 1988). It is also possible to be advantageous to both source plant and target insect (synomones).

The allomones that are known to prevent, block, or interfere with food selection and consumption by herbivorous insects are considered to be feeding inhibitors (Frazier & Chyb, 1995). The response of herbivorous insects to allomones is not restricted only to toxicity and disruption of the food selection and digestive process. Allomones can damage the physiology of an insect. They can act as nymphal growth retardants, antihormones or in extreme cases as insecticides. Plants advertise the presence of those substances by a signal of visual or olfactory nature. However insects sometimes reverse the repellent nature of these substances and use them as feeding attractants for food location (Harborne, 1993). For example mustard oils

which occur in crucifers, have a pungent acrid smell. However phytophagous insects – the cabbage and rape white butterfly *Pieris brassicae* (L.), the cabbage aphid *Brevicoryne brassicae* (L.), and the flea beetles *Phyllotreta* sp.– rely on mustard oils to select their host plants using them as phagostimulants.

There are a number of insects that have evolved adaptations not only to cope with these compounds but also to use them for their own benefit (protection or pheromone production). In some cases, insects can even sequester allelochemicals from plants to use them against their own natural enemies. For example the oleander aphid *Aphis nerii* (Boyer) can sequester alkaloids and cardenolides from both oleander *Nerium oleander* (L.) and milkweed *Asclepias curassavica* (L.) host plants (Rothschild *et al.*, 1970). Plant-derived cardiac glycosides isolated from the aphid were ingested by feeding preferentially from the internal primary phloem (Botha *et al.*, 1977). Ladybirds refused to feed on lupin aphids *Macrosiphum albifrons* (Essig.), which had fed on the more toxic quinolizidine alkaloids (Wink & Romer, 1986). However in another case, ladybirds were frequently found not only feeding on the aphid colonies but also sequestering the pyrolizidine alkaloids from the aphids which have been shown to sequester the pyrolizidine alkaloid pattern of their respective host plants (Witte *et al.*, 1990).

1.3 Plant stress and allelochemicals

Secondary plant metabolite synthesis and concentrations in plant tissue are affected by many environmental and genetic factors. Chemicals produced by a plant as a defence mechanism are often regarded to be reactions to stress. The toxin content of pest damaged plant tissue is often more likely to be higher than that in undamaged tissue (Young, 1991). Today it is believed that the major force in the development of plant-insect interactions is the synthesis of secondary metabolites by plants, than the presence of nutrients such as amino acids carbohydrates or vitamins itself (Berenbaum, 1986; Thorsteinson, 1960).

Environmental stress can reduce the performance of a plant and when it does, the suitability of the plant as a host to herbivorous insects may be altered (Heinrichs, 1988). Physiological changes which occur in a plant as a result of stress, often do not produce visible symptoms. In mineral-stressed plants, despite the absence of visible symptoms, the altered plant chemistry can have profound effects on their suitability as hosts for insects (Heinrichs, 1988). Contradictory results report that nitrogen deficiency of a host plant either increases, decreases or has no effect in the multiplication of the aphids (van Emden *et al.*, 1969). In certain cases it has been shown that nitrogen, phosphorous and potassium deficiency in the soil, increased proteolysis and enriched the soluble nitrogen content of plant sap resulting in increase of the aphid population. Where it has been shown that increased N dosages have increased the soluble N levels, the population growth of the aphids including the peach potato aphid *Myzus persicae* has also been increased (van Emden *et al.*, 1969).

Inducible plant factors that confer resistance to insects include those stimulated by various forms of environmental stress, as well as prior insect attacks on the plant (Norris, 1988). Limited information is available on insect attacks affecting subsequent plant responses to physical environmental stress. Green bug *Schizaphis graminum* (Rondani) feeding on drought-resistant winter-wheat *Triticum aestivum* (L.), increased cell membrane injury which rendered the plants less drought resistant (Dorschner *et al.*, 1986). Without greenbug feeding the wheat cell membranes were much more stable against solute leakage when subjected to the osmotic stress associated with a soil moisture deficiency.

1.4 Secondary plant metabolites and their role in aphid feeding behaviour

Aphids (Hemiptera: Homoptera, Aphididae) are of great economic importance because they damage plants by inoculating toxins and transmitting viral diseases through their saliva, by draining the plant's nutrients and by interfering with photosynthetic efficiency providing a rich medium for fungal growth through their

excretion products (Schepers, 1989). Their ability to avoid vacuole-sequestered plant toxins by moving their stylets intercellularly toward the relatively non-toxic sap flowing in the phloem, combined with parthenogenetic reproduction have made these insects one of the most successful groups of herbivorous pests.

After aphid alightment on a plant, the chemicals on the plant's surface play an important role in host acceptance or rejection. These are components of the epicuticular lipid layer of the plant (Dillwith & Berberet, 1990) or components of the trichomes of certain plant species (Ave *et al.*, 1987; Gibson & Pickett, 1983). Their nature is perceived by chemoreceptors located on the antennae (Bromley *et al.*, 1979) and on the apex of the tibiae and the tarsi (Anderson & Bromley, 1987) of the aphid. The next stage in evaluating the quality of a plant as a possible source of food is the probing and tasting of inner plant components, which are transferred mainly by gustatory papillae in the epipharyngeal organ (McLean & Kinsey, 1985; Wensler & Filshie, 1969). Once the aphid's stylet reaches its final feeding site, which in most cases is believed to be the phloem (Pollard, 1973), and provided that the insect does not encounter toxic compounds (Molyneux *et al.*, 1990), it will start reproducing. The aphid's reproductive performance depends on the nutritional value of the sap as assessed from its amino acid content (Wikteliuss *et al.*, 1990) and composition (Weibull, 1988).

Many studies performed by different researchers worldwide have examined the effects of secondary plant substances on various life parameters of different aphid species. It has been proved that some of these substances contribute to the plant's chemical defence whilst others act as feeding attractants. The role of some plant allelochemicals as natural feeding deterrents may be minimal, although their concentrations in certain plant tissue are high; the aphids in many cases are able to avoid them. In addition their high effective dose (ED50) values *in vitro* indicate that these substances would have to occur in unrealistically high concentrations in the phloem sap to act as defensive barriers. Deterrence (antixenosis) or antibiosis has been demonstrated for various alkaloids, glucosinolates, hydroxamic acids,

flavonoids, phenolics, coumarins and a variety of other compounds. Case studies relating to the effects of the aforementioned substances on aphids are presented in the following sections.

1.4.1 Indole alkaloids

Alkaloids are basic nitrogenous compounds that can form salts with acids and can be found widely in nature (Pettersen *et al.*). True alkaloids are mostly basic, contain nitrogen in a heterocyclic ring and derive from amino acid precursors. Pseudoalkaloids are usually basic not derived from amino acid precursors, while protoalkaloids are basic amines that have been derived from amino acids but their nitrogen is not a part of heterocyclic ring (Pettersen *et al.*). In general alkaloids are regarded to be part of a plant's chemical defence. In specific plants, alkaloids are found in a species-specific and genetically programmed accumulation pattern which remains unaffected by herbivory, microbial attack and mechanical damage or stress (Hartmann, 1991). Numerous alkaloids have been reported to be toxic or deterrent to insects (Bernays, 1998). Nicotine is one of the first insecticides which was used in agriculture. Deterency effects of alkaloids may also be related to their taste properties. Wounding-induced increase of alkaloid accumulation has been reported for indole alkaloids in *Catharanthus roseus* (= *Vinca rosea* L.) (Frischknecht *et al.*, 1987) and for quinolizidine alkaloids in lupine leaves (Wink, 1983). Zúñiga & Corcuera, (1986) and Zúñiga *et al.* (1985) suggested that gramine, an indole alkaloid present in barley *Hordeum* species, plays a role in the resistance of seedlings to *S. graminum* and the bird-cherry oat aphid *Rhopalosiphum padi* (L.). Older plants with lower gramine levels were more susceptible to infestation compared to younger ones. Gramine decreased the rate of feeding, survival and reproductive index of aphids feeding on artificial diets at concentrations similar to those found in plant leaves. Gramine concentration in barley is sufficiently high to cause toxicity or feeding deterrence to aphids. In addition Zúñiga *et al.* (1988), presented evidence that the presence of gramine in tissues other than the phloem affects subsequent feeding behaviour of *S. graminum* on barley. Argandoña *et al.* (1987), suggested that since gramine was not found in the vascular bundles of barley, it could be encountered by

the aphids during their search for phloem tissue.

Leszczynski *et al.* (1989), examining the contribution of indole alkaloids to the aphid resistance profile of winter wheat *T. aestivum*, found that indole alkaloids induced positive antibiosis effects on the cereal aphid *Sitobion avenae* (F.). The antibiosis effects of gramine were confirmed by Lohar (1989), who observed that when *S. graminum* or *R. padi* were fed artificial diets containing gramine, or allowed to feed on barley plants treated with this alkaloid, both species showed a significant increase in the duration of digestion; thus gramine plays an important role in the resistance of barley to aphids. Furthermore, Salas *et al.* (1990), found that nitrate fertilisation affects the performance of *S. graminum* on barley seedlings, because possibly it causes changes in gramine concentration in the leaves. In addition Salas & Corcuera (1991), suggested that barley seedlings grown under high temperatures and a long photoperiod are more resistant to aphids, because these environmental factors caused an increase in gramine concentration in the youngest leaves.

1.4.2 Quinolizidine alkaloids

The high content of lupins in quinolizidine alkaloids makes them resistant to aphids. In particular polyphagous aphids, such as the pea aphid *Acyrtosiphon pisum* (Harris) are able to colonise only lupins that contain low amounts of quinolizidine alkaloids. Lupine susceptibility was reduced when alkaloids were infiltrated into them (Wegorek & Krzymanska, 1971; Wegorek & Krzymanska, 1968). *M. albifrons* is an other example of an aphid species specialised in feeding on alkaloid rich lupins. Although the alkaloids ingested were found in the honeydew, the aphids sequestered them in concentrations that proved toxic to the predator carabid beetle *Carabus problematicus* in the laboratory (Wink & Romer, 1986). The aphid does not infest alkaloid-free plants and qualitative and quantitative patterns of the host plant are important in determining the infestation. In particular *M. albifrons* reared on yellow lupin *Lupinus luteus* (L.) in which sparteine and lupinine are the main alkaloids, showed the greatest reduction in development as compared with white lupin *Lupinus albus* (L.) and tarwi lupin *Lupinus mutabilis* (L.) where the main alkaloid is lupanine

(Emrich & Schmutterer, 1990). On narrowleafed lupin *Lupinus angustifolius* (L.) the nymphal development was shortest and the reproductive period longest but average daily nymphal production was lower than on the other lupins. However earlier work performed by Smith (1966), proved that sparteine had a phagostimulant effect for the aphid *Acyrtosiphon spartii* (Koch), fed on the broom *Cytisus* = (*Sarothamnus*) *scoparius* (L.). This aphid migrates within developing plants, following sparteine-rich tissue. Recently Berlandier (1996), examined the effects of alkaloid levels of *L. angustifolius* on the performance of two clones of the aphid *M. persicae*. Ingestion of artificial diet containing 80µg/ml or greater of *L. angustifolius* extract reduced feeding and increased mortality. However it was not elaborated if the aphid mortality was due to toxic effects of the alkaloids or due to starvation (alluding to feeding deterency). The alkaloids appeared to have a stronger influence on the aphids when presented in an artificial diet than *in situ*.

Dreyer *et al.* (1985), compared the effects of different alkaloid groups on *A. pisum* aphids. They found that an indolizidine alkaloid known as castanospermine, was intensely deterrent as were quinolizidine alkaloids, but only modest feeding deterency was observed with most of the pyrolizidine alkaloids tested. The broom aphid *Aphis cytisorum* (Hartig) appears to prefer to feed on *Lupinus* and *Cytisus* species having a relatively high alkaloid content. This aphid sequesters quinolizidine alkaloids from the flowering stems of the broom *C. scoparius*; stems contain elevated amounts of alkaloids compared to other parts of the plant (Wink *et al.*, 1982). However the aphid tends to infest only those plants that have an alkaloid content below average. The composition of the alkaloid mixture in the aphids was similar but not exactly the same as that found in the plants.

1.4.3 Hydroxamic acids

The poor population development of the rose-grain aphid *Metopolophium dirhodum* (Walker), corn leaf aphid *Rhopalosiphum maidis* (Fitch) and *S. graminum* in the early developmental stages of certain cereals has been attributed to the elevated levels of hydroxamic acids detected in the plant tissue (Argandoña *et al.*, 1983;

Argandoña *et al.*, 1980). Like gramine in barley, the levels of hydroxamic acids in wheat and maize decreased with the age of the plant tissue, resulting in heavy aphid infestations on older leaves; younger plants and leaves were less infested (Argandoña *et al.*, 1981; Argandoña *et al.*, 1980).

When DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) -the main hydroxamic acid in wheat and maize extracts- was added to artificial diets, it induced antibiotic and antifeedant effects on various cereal aphids (Corcuera *et al.*, 1985; Argandoña *et al.*, 1983; Zúñiga *et al.*, 1983; Argandoña *et al.*, 1981; Argandoña *et al.*, 1980; Long *et al.*, 1977). The feeding deterrent effect on aphids reared on plants containing high DIMBOA levels was indicated by reduced weight gain and honeydew production; aphids reared on plants containing low DIMBOA levels suffered less. The most susceptible aphid proved to be *S. graminum* and the least *R. maidis*. In addition, infiltration of DIMBOA into barley leaves lacking hydroxamic acids, induced a negative correlation between the levels of DIMBOA and the infestation numbers of *M. dirhodum*. Furthermore the 2-O- β -D glucoside of DIMBOA present in uninjured plants, was less active than DIMBOA itself (Corcuera *et al.*, 1985).

1.4.4 Glucosinolates

Sinigrin, which is a mustard oil glycoside that occurs in brassicas, affects the host selection of the aphid *B. brassicae*. Sinigrin-treated leaves of the non-host broad bean *Vicia faba* (L.) were apparently preferred to untreated leaves by the aphid *B. brassicae*. Sinigrin increased probing and the duration of probes of the aphid (Wensler, 1962). Sinigrin is considered to be a specific stimulus for host selection by *B. brassicae* but is detected only after the stylets have penetrated the leaf (Wensler, 1962). When sinigrin was incorporated into a diet containing sucrose, it rendered the diet more acceptable to *B. brassicae* (Moon, 1967). Schoonhoven & Derksen-Koppers (1976), showed that sinigrin and the glycoalkaloid tomatine increased the acceptability of an artificial diet given to the aphid *M. persicae*. However it was later found (Qin Junde & Ke Lidao, 1984) that sinigrin made artificial diets less

acceptable to a Beijing clone of *M. persicae*. *B. brassicae* and *M. persicae* showed different strategies in dealing with glucosinolates ingested: while the former retained high concentrations of glucosinolates in the body, the latter excreted high concentrations in the honeydew (Weber *et al.*, 1986).

Nault & Styer (1972), examined the feeding response of six aphid species to sinigrin. This glucosinolate was found to be phagostimulant for the turnip aphid *Hyadaphis* = (*Lipaphis*) *erysimi* (Kaltenbach) and *B. brassicae*, both important pests of *Cruciferae*. Two polyphagous species, the black bean aphid *Aphis fabae* (Scopoli) and the foxglove aphid *Acyrtosiphon* = (*Aulacorthum*) *solani* (Kalt.), which are not pests of the *Cruciferae*, and the oligophagous *A. pisum* which is restricted to *Leguminosae*, were deterred by sinigrin-treated broadbean leaves. *M. persicae* which feeds widely among the *Cruciferae*, was stimulated to feed on sinigrin-treated bean leaves. All the clones of *M. persicae* fed on broadbeans treated with a high concentration of sinigrin. This treatment stimulated feeding and larviposition of *M. persicae*. Preconditioning of *M. persicae* to a host (turnip) containing mustard oil glycosides was not necessary for aphids to respond to sinigrin-treated leaves. *M. persicae* reared on potato and chrysanthemum also fed on sinigrin-treated broadbean leaves compared to non-treated leaves. The addition of sinigrin to leaves probably does alter leaf physiology in some way, possibly in the breakdown of proteins, etc., and aphids in part may be responding to these changes. The marginal response of *M. persicae* to treated leaves may be due solely to these changes rather than to phagostimulatory effects of sinigrin.

In addition Klingauf & Nocker-Wenzel (1972), found that the acceptance or refusal by aphids of cruciferous plants corresponded to their reaction to sinigrin *in vitro*. Aphids such as *B. brassicae* and *M. persicae* which have glucosinolate-containing crucifers as hosts, were more stimulated by sinigrin to feed on a sucrose-containing diet than *R. padi*, *A. fabae*, and *A. pisum*, for which crucifers are non-hosts. Additional evidence however proved that sinigrin in artificial diets could be repellent to *M. persicae* (van Emden, 1972; Wearing, 1968). On brassicas, *M. persicae* occurs

mainly on the older leaves but *B. brassicae* is found on younger leaves where the mustard oil levels are higher (van Emden, 1971). It has been proved that *B. brassicae* and *H. erysimi* which live on cruciferous plants rich in sinigrin, have an enzyme in their tissue (glucosinolase) capable of detoxifying sinigrin (MacGibbon & Benzenberg, 1978).

Glucosinolates proved to have antibiotic effects against *H. erysimi*. They decreased survival in diets (Dilawari & Atwal, 1987) and positive correlations were found between total glucosinolate content of various crucifers and resistance to this aphid (Gill & Bakhetia, 1985; Malik, 1981).

1.4.5 Flavonoid and phenolic compounds

High concentrations of the flavonoids catechin and epicatechin observed in the late buds and flowers of rose, induced feeding deterency to the rose aphid *Macrosiphum rosae* (L.) (Miles, 1978). This aphid walked off the flowers as the sepals opened. In addition Zucker (1982), suggested that phenolic compounds play an important role in habitat selection by the galling aphid *Pemphigus betae* (Auct.). Total phenols were negatively correlated with the suitability for galling of a tree, a leaf, or a leaf section at the time of bud burst in the narrowleaf cottonwood *Populus angustifolia*.

The phenolic compound phlorizin, which is frequently found in apples is not a feeding stimulant for the green apple aphid *Aphis pomi* (De Geer). However it was a probing deterrent to the non-apple feeding aphids *M. persicae* and the North American aphid vector of the raspberry mosaic virus *Amphorophora agathonica* (Hottes). Phlorizin was an ingestion deterrent for all of the aphid species examined. The *M. persicae* feeding deterency was later confirmed by Qin Junde & Ke Lidao (1984), who showed that the addition of phlorizin to artificial diets reduced the diet uptake by this aphid. The fact that apple is utilised as a host by *A. pomi* was associated with its feeding in the phloem which appeared not to contain phlorizin (Montgomery & Arn, 1974).

1.4.6 Other compounds

Various plant allelochemicals have been tested in artificial diets for effects on feeding preference and performance of several aphid species. (Schoonhoven & Derksen-Koppers, (1976) showed that food selection by a polyphagous aphid such as *M. persicae*, is primarily due to the presence of nutrients in the sap in combination with the absence of feeding deterrents. By adding 24 secondary plant substances to artificial diets in dual choice experiments, they found that none of these compounds increased diet acceptability. With the exception of sinigrin and tomatine, all compounds made the diet less acceptable. Qin Junde & Ke Lidao (1984), showed that the addition of secondary plant substances to *M. persicae* artificial diets caused reductions of uptake to different degrees in the order: nicotine > phlorizin > gossypol > tomatine > malic acid > inulin > sinigrin. None of these compounds showed phagostimulant effects. The addition of inulin, malic acid or sinigrin did not cause a significant difference in the rate of larval development or in adult longevity. Diets containing these substances could support the aphids' development and reproduction for at least three generations. Smith (1966) suggested that the principal defence of legumes to aphids is the possession of secondary plant compounds including phenolics, alkaloids and polysaccharides. The occurrence and distribution of these compounds are determinants of aphid-legume specificity. Two more examples of feeding deterrence effects that secondary plant substances can induce on aphids are associated with hydrogen cyanide and coumarins. Cyanide present in cyanogenic plants of white clover *Trifolium repens* (L.) makes them less susceptible to black lucerne aphid *Aphis craccivora* (Koch) compared to acyanogenic phenotypes of clover (Dritschilo *et al.*, 1979). Coumarins present in *Lupinus termis* (= *L. albus*) reduced its acceptability by *A. craccivora* compared to that of *V. faba*. Systemic or topical application of coumarin on *V. faba* diminished the performance of the aphid (Mansour *et al.*, 1982).

From the examples mentioned above, it is concluded that secondary plant substances play a major role in defensive strategies against aphids. These chemicals can impair the normal physiology of the insect in a variety of ways as larval growth retardants,

feeding deterrents, antihormones, or in extreme cases as insecticides. However in certain cases their role has been reversed by aphids which use them as feeding stimulants or feeding attractants. It is unknown whether the same allomones are present in phloem sap as in the mesophyll cells. In spite of the abundant literature on host plant composition and insect development little information is available on the detection of allomones in phloem in relation to aphid development (Wink *et al.*, 1982; Dixon, 1975; Eschrich, 1970). However some defensive chemicals like the non-protein amino acid canavanine and phenols have been recorded in phloem sap (Zeigler, 1975; Dixon, 1975).

1.5 Glycoalkaloids

The potato *Solanum tuberosum* (L.) is considered an essential crop contributing to the human diet in many parts of the world. Potatoes provide an excellent source of carbohydrates and contribute to our daily requirement of vitamin C (Jadhav *et al.*, 1981). Significant amounts of minerals like phosphorous, potassium and calcium can be provided through potato consumption. Potato plants can be grown plentifully under moderate climate and their tubers can be subjected to long transportation and storage. Total potato production and consumption of raw and processed potato products (crisps, flakes, tinned potatoes) has been increasing in developed and developing countries over the last twenty years (Salunkhe & Kadam, 1991). Potatoes, like most crop plants, produce secondary metabolites known as glycoalkaloids (GAs). Glycoalkaloids are toxic nitrogenous steroid glycosides, which derive from a steroid alkaloid (aglycone part) combined with a sugar (a carbohydrate moiety). Steroid alkaloids occur in *Solanaceae*, *Liliaceae*, *Asclepiadaceae* (Heftmann, 1983).

The most important steroid alkaloids (aglycones) in tuber-bearing solanaceous plants are solanidine, demissidine, acetylleptinidine, tomatidine and tomatidenol (Salunkhe & Kadam, 1991). The different forms of glycoalkaloids present in wild relatives of potato found in South America derive from the combination of these aglycones with

a different carbohydrate moiety. Solanine was first reported by Baup 1826 cited in Jadhav *et al.* 1981. The term solanidine is attributed to Zwenger and Kind 1861 who found that solanine is actually a glycoside and referred to the alkaloid as solanidine cited in (Jadhav *et al.*, 1981). Much later Kuhn & Low (1954), identified the presence of α -chaconine in the foliage and the shoots of the wild *Solanum chacoense* (Bitt.). The differences in the chemical composition of α -solanine and α -chaconine are related to the composition of their carbohydrate moiety (Nair *et al.*, 1981) (Table 1.1).

Table 1.1. Occurrence and composition of α -, β - and γ - forms of solanine and chaconine found in Solanaceae (after Rayburn *et al.*, 1994; Salunkhe & Kadam, 1991).

Glycoalkaloid	Aglycone	Sugar moiety
α -solanine	Solanidine	β -D -galactose, β -D -glucose, α -L-rhamnose
β 1-solanine	Solanidine	β -D -galactose, β -D -glucose
β 2-solanine	Solanidine	β -D -galactose, α -L-rhamnose
γ -solanine	Solanidine	β -D -galactose
α -chaconine	Solanidine	β -D-glucose, α -L-rhamnose, α -L-rhamnose
β 1-chaconine	Solanidine	β -D-glucose, α -L-rhamnose
β 2-chaconine	Solanidine	β -D-glucose, α -L-rhamnose
γ -chaconine	Solanidine	β -D-glucose
D and L refer to the stereoisomer form of the sugar		

In α -chaconine, the aglycone part is the steroid solanidine and the sugar moiety which is linked to the steroid consists of glucose-rhamnose-rhamnose. In α -solanine, solanidine is the aglycone and galactose-glucose-rhamnose compose the sugar moiety. Usually in most potato cultivars α -solanine represents approximately 40% of the total glycoalkaloids in plants and α -chaconine around 60% (Guseva *et al.*, 1960). In commercially available potato tubers destined for human consumption, 95% or more of the total glycoalkaloid (GA) fraction consists of α -solanine and α -chaconine (Jadhav *et al.*, 1981). Besides these main glycoalkaloids the remaining 5% consist of β - and γ - forms of solanine and chaconine possessing a shortened chain. β - and γ - forms are composed of the aglycone solanidine bonded with two and one parts respectively of the relevant sugar part of the α - forms (Table 1.1). The percentage of existing β - and γ - forms is strongly dependent on the potato cultivar. For example Zitnak (1961), reported that peels of cv Netted Gem contained high solanidine concentrations. In addition he detected β -chaconine in peel of cvs Sebago

and Kennebec in concentrations up to 30% of total glycoalkaloid concentration.

1.6 Distribution of glycoalkaloids in the potato plant

Glycoalkaloids can be detected in most parts of the potato plant including tubers, eyes and sprouts, stems, leaves, flowers (Jadhav *et al.*, 1981; Lampitt *et al.*, 1943) and fruits (Coxon, 1981). Table 1.2 shows the distribution of total glycoalkaloids in the potato plant and various tuber tissues (Wood & Young, 1974).

Table 1.2. Distribution of total glycoalkaloids in the potato plant and various tuber tissues (After Wood and Young, 1974)

Potato part	Total glycoalkaloids (mg/100g fresh weight)
Plant	
Sprouts	200-400
Flowers	300-500
Stems	3
Leaves	40-100
Normal tuber tissue	
Skin, 2-3% of tuber	30-60
Peel, 10-15% of tuber	15-30
Peel and eye, 3mm disk	30-50
Peels from bitter tubers	150-220
Flesh	1.2-5
Whole tuber	7.5
Bitter tubers	25-80

Glycoalkaloids are formed in the parenchyma cells of the periderm and cortex of the tubers (Reeve *et al.*, 1969), and their concentration is high in regions of the plant showing high meristematic activity such as eye regions, leaf buds and young leaves (Hilton, 1951). Glycoalkaloids have also been detected in tissue culture of *S. tuberosum in vitro* (Khanna *et al.*, 1998). The glycoalkaloid content of *S. tuberosum* is highest in the flowers (Morgenstern, 1998; Lampitt *et al.*, 1943) and in the tubers it is concentrated in the peel, sprouts and in areas around the eyes (Lampitt *et al.*, 1943) cited in Jadhav *et al.* (1981). Commercial potatoes generally have foliar total glycoalkaloid levels between 40-100 mg/100g fresh weight (FW), and tuber total glycoalkaloid levels between 2-10mg/100g FW. The glycoalkaloid content in potato sprouts ranges from 200-400mg/100g FW. The total glycoalkaloid content of foliage

and tubers is highly correlated (Deahl *et al.*, 1973). Paseshnichenko (1957), showed that in the leaves and tubers a decrease in glycoalkaloid content affected both α -solanine and α -chaconine during the vegetative process. However a decrease in the glycoalkaloid content of seedlings was at the expense of α -chaconine only.

The subcellular distribution of potato glycoalkaloids has been investigated by Roddick (1977). Cell fractions of potato and tomato organs were examined after homogenisation and differential centrifugation. In both species concentrations of glycoalkaloids were found to accumulate mainly in the soluble fraction and smaller amounts were found in the microsomal fraction. Glycoalkaloids appeared sporadically in the mitochondrial fractions and were rarely detected in lower fractions. As glycoalkaloids were present in the microsomal phase, synthesis may occur in the microsomes and possibly are then transported to the soluble phase of the cell for storage. Although glycoalkaloids were concentrated in the soluble phase it is not known whether they accumulate mainly in the vacuole or the soluble phase of the cytoplasm. However Roddick (1982), considers that although glycoalkaloid transport between root and shoot does not take place in potato, the transport of these substances is possible at the subcellular level, especially between the site of synthesis/glycosylation (probably particulate) and the site of accumulation (probably vacuolar).

1.7 Biosynthesis of glycoalkaloids

The toxic glycosidic alkaloids contained in potato *S. tuberosum* derive biosynthetically from cholesterol. Solanidine is considered to be pseudoalkaloid because it does not derive from amino acid precursors. Only the very basic information from the extensive studies performed on glycoalkaloid origin and biosynthesis are presented here since a detailed description of glycoalkaloid biosynthesis is far from the aim of this thesis. All steroid compounds such as sterols, certain sapogenins, terpene hormones and alkaloids are interrelated in terms of the ways they are synthesised in the plant genera in which they are found. The regular

pathway starting from acetate via mevalonate, isopentenyl pyrophosphate, farnesyl pyrophosphate, squalene, and cholesterol ending in the synthesis of solanidine is applicable to steroid alkaloids (Jadhav & Salunkhe, 1975). Graphic representation 1.1 shows the basic biosynthetic path of glycoalkaloid synthesis after Jadhav & Salunkhe, (1975).

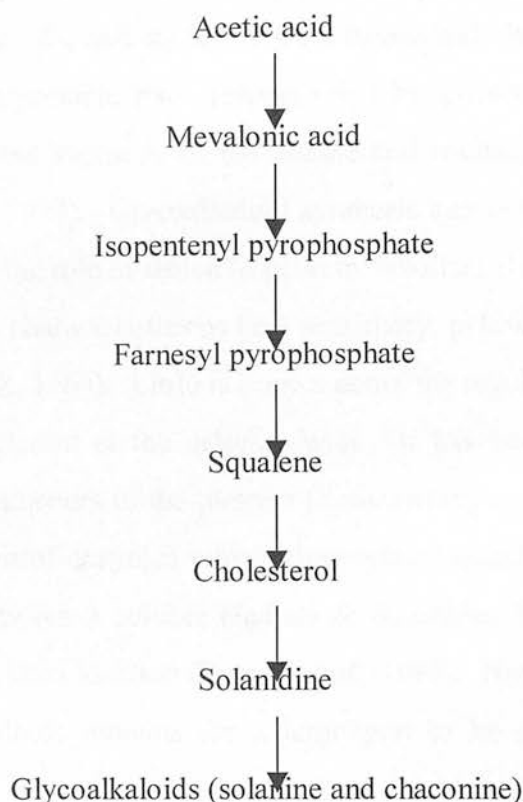


Figure 1.1. The biosynthetic path of glycoalkaloid synthesis after Jadhav & Salunkhe, (1975).

First Guseva & Paseshnichenko (1958), demonstrated the uptake and utilisation of radioactive acetate by potato sprouts. The glycoalkaloids isolated from such sprouts grown under conditions of normal illumination had the labelled carbon mainly in the aglycone while the labelled carbon of sprouts grown in the dark was in both the aglycone and the sugar portions of the glycoalkaloids. Furthermore Guseva *et al.* (1960), reported the utilisation of radioactive acetate and mevalonate for the synthesis of glycoalkaloids in potato sprouts and leaves. Wu & Salunkhe (1978), observed that the percent incorporation of a label from mevalonate into α -solanine in light-exposed tubers was more than that of mechanically injured tubers. Johnson *et*

al. (1964), established the biosynthesis of mevalonic acid to cholesterol by isolating radioactive cholesterol from *S. tuberosum* fed with mevalonic acid-2-¹⁴C. Tschesche & Hulpke (1967), reported that cholesterol was metabolised to solanidine when applied to leaf surfaces of potato plants.

In vitro synthesis of γ -, β -, and α - forms of solanine and chaconine (one two and three sugars in the glycosidic part, respectively) by potato tissue supported the hypothesis of a stepwise synthesis of α -solanine and α -chaconine from solanidine (Osman & Zacharius, 1979). Glycoalkaloid synthesis and hydrolysis are controlled by an enzymic system the role of which in plant metabolism is not clearly understood and depended on such characteristics as heat sensitivity, pH, water extractability and light sensitivity (Zitnak, 1964). Little is known about the regulation of glycoalkaloid synthesis and accumulation at the cellular level. It has been suggested that the synthesis of solanidine occurs in the plastids (Ramaswamy *et al.*, 1976). Studies of the subcellular location of enzymes which glucosylate steroid alkaloids have so far only distinguished between a soluble (Jadhav & Salunkhe, 1973; Liljegren, 1971) and a membrane-associated location (Osman *et al.*, 1980). However the biosynthetic pathway to glycoalkaloids remains for a large part to be elucidated (Heftmann, 1983).

1.8 Glycoalkaloid synthesis as affected by different factors

1.8.1 Photoinduction

Potato tubers exposed to light in field conditions or during postharvest handling and marketing develop a green pigmentation of the surface. This “greening” indicates that chlorophyll has been formed; however, chlorophyll is harmless and tasteless. Green potatoes are usually associated with increased levels of glycoalkaloids, although the chlorophyll and glycoalkaloid formation are two different processes independent of each other (Jadhav *et al.*, 1981).

However light is well documented as an elicitor of glycoalkaloid synthesis (Sinden,

1972; Zitnak, 1961; Gull & Isenberg, 1960; Conner, 1937). The chances that tubers will be exposed to light of different intensity and unpredictable duration -ranging from daylight to ultraviolet, fluorescent or even incandescent light- vary with environmental factors and marketing conditions and it has proved a major problem. However (Percival, 1993) concluded that glycoalkaloid synthesis is possible in the dark following exposure to light and that tubers are most responsive to light treatment during the first few weeks of storage. In addition, he suggested that low storage temperature (5°C) is important to maintain low glycoalkaloid concentrations in potato tubers.

1.8.2 Cultivar

Glycoalkaloid synthesis is influenced by several environmental factors but most important is plant genotype (Salunkhe & Kadam, 1991). Since the centre of origin of the potato is considered to be South America, many wild tuber-bearing *Solanum* species and most cultivated South American species have been screened for potential use, or they are already used in potato breeding (Gregory, 1981; Sinden *et al.*, 1980). However high concentrations of glycoalkaloids existing in parental wild species can be transmitted to hybrid progeny. In 1970 the newly developed cv. Lenape was found to contain unacceptable tuber glycoalkaloid concentrations and was withdrawn from the market. Lenape was derived from *S. chacoense*, a wild potato species with extremely high glycoalkaloid concentrations in its ancestry (Zitnak & Johnston, 1970). The same authors recorded glycoalkaloid concentrations up to 35mg/100g FW in cv Lenape resulting in withdrawal of the cultivar from commercial cultivation. The range of glycoalkaloid concentrations in commercial potatoes is usually 1.0-15mg/100g FW, well below the acceptable maximum of 20mg/100g FW recommended for food safety (Gregory, 1984).

Glycoalkaloid content of potato cultivars is largely under genetic control (Sinden *et al.*, 1984). If wild potato species are to be used in a breeding programme, glycoalkaloid assessments should be performed and given high priority. According to Gregory (1984), tuber-bearing *Solanum* species exhibit a broad spectrum of

glycoalkaloid content. Some species of interest to breeders owing to their tolerance of insect attacks or water deficiency, may contain extremely high total glycoalkaloid levels, or unusual types of glycoalkaloids of ill-defined or unknown toxic and teratogenic properties. Unacceptable increases in tuber glycoalkaloid concentrations could occur from the use of certain wild *Solanum* species in breeding programs (Gregory, 1984). The occurrence of this wide quantitative and qualitative diversity of alkaloids in potatoes may have emerged through hybridisation of such species as *S. chacoense* and *Solanum demissum* (Lindl.). For example, the incorporation of wild germplasm into a breeding programme introduced two major glycoalkaloids known as demissine and commersonine derived from the parental lines of *S. chacoense* and *Solanum commersonii* (Dun.) respectively (Osman *et al.*, 1976).

Furthermore high correlations have been identified between glycoalkaloid concentrations in foliage and tubers. Deahl *et al.* (1973), concluded that for a sample of potato clones, tuber glycoalkaloid contents and foliar glycoalkaloid contents were highly correlated. This conclusion is in agreement with the results obtained by Schwarze (1962, 1963) who suggested that there is a high correlation between levels of alkaloids in the foliage and in the tubers. However the selection of types with high foliage levels and low tuber levels is possible but their frequency is low. Although high correlation between glycoalkaloid concentrations in foliage and tubers have been identified, relatively little research has been done describing the foliar glycoalkaloid concentrations of wild *Solanum* spp. This information is considered highly important owing to the increased use of wild *Solanum* spp. in breeding programmes (Tingey *et al.*, 1978).

1.8.3 Location, climate and environment

Although the level of glycoalkaloid synthesis in potato is genetically determined, there can be large fluctuations in the glycoalkaloid levels resulting from different environmental influences. The location of the potato crop -as related to temperature, altitude, light exposure and photoperiod- was found to be correlated with glycoalkaloid formation. Sinden & Webb (1972), reported significant differences in

tuber glycoalkaloid concentrations in five commercial cultivars grown in 39 locations in the USA. These differences were attributed not only to the different genetic origin of the cultivars grown, but also to the effects of the crop location.

In the UK, Bintcliffe *et al.* (1982), found that glycoalkaloid concentration was affected by cultivar, site and season of growing. In addition, they found significant interactions between cultivar x site and cultivar x season. The tuber glycoalkaloid concentrations ranged from 3.6 to 14.2 mg/100g FW. They concluded that glycoalkaloid screening of new cultivars should be performed in different sites and seasons. However Zitnak (1955) cited by Jadhav *et al.* (1981), observed that the influence of locations was of an insignificant nature. The slightly higher levels of glycoalkaloids in potatoes associated with certain locations were considered to be a result of conditions during transportation of the tubers. The variability in the glycoalkaloid concentrations in certain cultivars was attributed to climatic environmental variations rather than to the influence of soil conditions in different locations. Jadhav *et al.* (1981), concluded that the conflict existing in the literature regarding the possible effects of location on glycoalkaloid production is probably due to the nature of the physiological stresses imposed by location, climate and environment leading to variations in the total glycoalkaloid content.

Hutchinson & Hilton (1955), concluded that differences in cultural practices applied to potato cv Netted Gem like planting depth, level of hilling, date of planting with single-eye and multiple-eye pieces as well as fertiliser rate were not associated with differences in solanine content. Although there was no greening of tubers, further investigation showed that the high glycoalkaloid levels appeared when one or more unfavourable environmental conditions like nutritional unbalance, frost or hail damage to the potato tops had prevailed before tuber maturity. In addition, soil moisture and water availability, through the method of irrigation (Gosselin *et al.*, 1988), have been shown to affect the glycoalkaloid concentrations of potato tubers. Morris & Petermann (1985), reported that hot and dry weather conditions during cultivation increased glycoalkaloid concentrations by approximately 60% compared

to those concentrations detected in potatoes grown in cooler and higher altitudes or coastal temperate climates in Australia.

Among the nitrogen-based toxicants, alkaloids as a class are considered to be the ones most affected by changes in the nutrient status of soils. It is possible to produce two- to 10-fold increases of alkaloids when plants are fertilised with high levels of nitrogen. Nowacki *et al.* (1976), demonstrated that generally plants that produced alkaloids originating from amino acids react to increased nitrogen fertilisation with added alkaloid synthesis. High levels of nitrogen fertilisation, increased glycoalkaloid concentrations in cultivars tested by Cronk *et al.* (1974). However Nowacki *et al.* (1975), indicated that increased nitrogen fertilisation resulted in lower foliar glycoalkaloid concentrations. Sewage sludge application to soils did not affect the total glycoalkaloid concentrations of potato tubers cv. Katahdin (Mondy *et al.*, 1984). However, when sodium molybdate was applied to the soil at 2.75, 6.75 and 10kg/hectare one day before planting potatoes cv. Katahdin, a significant decrease was induced in the glycoalkaloid concentrations (Munshi & Mondy, 1988). The greatest decrease occurred at the highest application rate of sodium molybdate.

Speroni *et al.* (1981), reported that ozone induced a significant ($P < 0.05$) reduction in the total FW glycoalkaloids. However the absolute changes estimated on a dry weight basis were non-significant ($P > 0.05$). As a result, the authors rejected the hypothesis that chemically induced foliar injury caused by the air pollutant ozone would increase the total glycoalkaloids in tubers. Higher levels of glycoalkaloids were associated with environmental factors that tend to retard the plant maturation process (Sinden & Webb, 1972). Usually immature and small tubers show more increases in glycoalkaloids on exposure to light than older and larger ones. Wolf & Duggar (1946), noticed an inverse relationship between tuber size and solanine concentration in the potato cvs White Rural and Irish Cobbler. However no correlation was found between time of maturation and solanine content in another 32 potato cultivars.

1.8.4 Physiological stress

Mechanical damage can affect the glycoalkaloid content of potato tubers. Elevated glycoalkaloid levels due to mechanical damage can be found in tubers subjected to increased mechanisation at harvest and during post harvest handling. Impact damage such as bruising and black spot (internal types of damage) and splits and cracks (visible on the tuber surface) were frequently found (Olsson, 1989).

The production of glycoalkaloids around the wound sites of potato tubers was initially reported by (McKee, 1955). Injury of tubers sustained by either bruising or mechanical grading after harvesting induced glycoalkaloid synthesis (Sinden, 1972). Mondy *et al.* (1987), reported that following bruising, glycoalkaloid concentrations in cv Katahdin increased over a 12 week period; greater accumulation of glycoalkaloids was observed during storage at 20°C. In addition Wu & Salunkhe (1976), reported that mechanical injuries like brushing, cutting, dropping, puncturing and hammering significantly stimulated glycoalkaloid synthesis in both peel and flesh of tubers. The same authors concluded that the relationship between glycoalkaloid concentration and mechanical damage is dependent upon cultivar, type of mechanical damage, temperature of storage and duration of storage. High storage temperature (21°C) stimulated glycoalkaloid synthesis more than low temperature (4°C). In addition, the highest degree of injury induced glycoalkaloid synthesis within 15 days.

Although the glycoalkaloids are localised in the peel of whole tubers, mechanical injury caused by slicing could increase their synthesis and accumulation in peeled tubers (Allen & Kuc, 1968). Extended research performed proves the correlation of potato slicing and elevated glycoalkaloid production (Ahmed & Müller, 1978; Salunkhe *et al.*, 1972). Compared to light and mechanical injuries, hollowheart and blackheart disorders are considered to be less potent factors stimulating glycoalkaloid synthesis in potato tubers (Jadhav *et al.*, 1980). However potato cultivars differ markedly with respect to the rate of glycoalkaloid formation in response to wounding which may be significant as a part of a disease-resistance mechanism. It is known

that older leaves of the potato plant contain a lower concentration of glycoalkaloids. Jadhav *et al.* (1981), suggested that the increased susceptibility of the older leaves to damage induced by an early-blight attack, may be due to the potato glycoalkaloids being involved in the temporary resistance of leaves to the development of the infestation.

1.9 Pharmacology and toxicology

Glycoalkaloids are of concern to human health because consumption of potatoes containing elevated amounts of glycoalkaloids has been repeatedly reported to induce disorders in humans and animals, including gastrointestinal disturbances, nervous depression and on some occasions death (Willmot, 1933). Excessive tuber total glycoalkaloid content has been associated with undesirable flavour and occasionally human illness, due to poisoning. Morris & Lee (1984), reviewed up to 30 deaths and over 2000 documented cases of glycoalkaloid poisoning involving consumption of potatoes in addition to numerous cases of livestock losses. According to the British Medical Journal (Anonymous, 1979), most mild and even severe cases of glycoalkaloid poisoning would be diagnosed as gastro-enteritis.

Because of their nitrogenous structure, many alkaloids interfere with essential components of the nervous system, particularly with the action of the chemical neuro-transmitters (i.e. acetylcholine, epinephrine, dopamine, etc) (Nowacki *et al.*, 1976). The current maximum residue limit for glycoalkaloids in fresh potatoes intended for human consumption is 20 mg/100g of fresh weight or 1000mg/kg of dry weight, assuming 20% dry matter. Glycoalkaloids have been described on a quantitative base as the most highly consumed natural toxin in the North American diet (Mackenzie & Gregory, 1979) because the consumption of potatoes averages almost 70 kg/year/person. These substances are not destroyed during food processing like baking, boiling or frying even at high temperatures (Bushway *et al.*, 1980).

Their main mode of action is by inhibiting cholinesterase activity (Roddick, 1989; Bushway *et al.*, 1987) thereby blocking nerve transmission, and by inducing hepatic ornithine decarboxylase (Caldwell *et al.*, 1991). In addition, they have been reported to be membrane disruptive (Fewell *et al.*, 1994; Roddick *et al.*, 1988; Roddick & Rijnenberg, 1986) and teratogenic (Keeler *et al.*, 1991; Morris & Lee, 1984) agents. In UK an outbreak of glycoalkaloid poisoning in school children was reported by McMillan & Thompson (1979). Symptoms of glycoalkaloid poisoning generally include gastrointestinal and neurological disturbances (cholinesterase activity inhibition) (Bushway *et al.*, 1987) with death being caused by central nervous system depression (Morgan & Coxon, 1987). Haemolytic and haemorrhagic damage can also occur in both the gastrointestinal tract and the retina. Teratogenic effects including skeletal damage as exhibited in the condition known as 'spina bifida' which has been attributed to over-consumption by pregnant women of potato tubers containing solanine (Harborne, 1993). When enormously large amounts of solanine accumulate in tubers that have been exposed above the soil surface and have become 'greened', death from solanine poisoning is a reality. Victims have no time to adapt to dealing with the toxin and, unless they are sick, they die from respiratory failure (Harborne, 1993).

1.10 Effects of *Solanum* glycoalkaloids on the major potato pests

Early studies implicated glycoalkaloids as factors in the resistance of potato plants towards fungal pathogens (Fewell *et al.*, 1994; Fewell & Roddick, 1993; Sinden *et al.*, 1973; Arneson & Durbin, 1968; Allen & Kuc, 1968; McKee, 1955; Agerburg *et al.*, 1933) and insects (Dahlman & Hibbs, 1967; Sturckow & Low, 1961; Pierzchalski, 1957; Schreiber, 1957). In his review of secondary plant metabolites for protection against pathogens and herbivores Wink (1988), refers to the potato as an example of a crop plant in which alkaloids have been eliminated from the tubers but maintained in the green parts so that some natural resistance to enemies is retained. The inhibitory effects of glycoalkaloids on both fungal and insect pests of the potato indicate that their evolutionary significance is most likely to be as natural

pesticides (Jadhav *et al.*, 1981).

An example of the role of *Solanum* sp. glycoalkaloids as contributors to natural defence in the potato is the case of the potato leafhopper *Empoasca fabae* (Harris). Dahlman & Hibbs (1967), concluded that solanine, solanidine and demissidine reduced the rate of initial imbibition, but not leafhopper survival, while leptine I markedly reduced both the imbibition rate and leafhopper survival. The main glycoalkaloid of tomato plants, tomatine, completely inhibited imbibition and increased the rate of nymphal mortality of potato leafhopper; however, its aglycone, tomatidine, did not affect the rate of imbibition or leafhopper survival for several hours. Tingey *et al.* (1978), suggested that the significant correlation found between foliar concentrations of total glycoalkaloid levels and potato leafhopper resistance indicates that glycoalkaloids may be an important factor in the defence of wild potato species against this pest. Feeding studies of total glycoalkaloid extracts from 10 wild tuber *Solanum* species, exhibiting different levels of resistance to *E. fabae*, provided additional evidence for a relationship between total leaf glycoalkaloid content and resistance to potato leafhopper. In particular Raman *et al.* (1979), showed that nymphal survival and duration of settling, salivation-ingestion, and non-feeding of potato leafhopper were significantly correlated with glycoalkaloid fractions extracted from the foliage of these wild potato species, which exhibited different levels of resistance to *E. fabae*.

Five *Solanum* genotypes, at various growth stages, which contained different percentages of glycoalkaloids, were found to be implicated in resistance to *E. fabae* (van de Klashorst & Tingey, 1979). Depending on the growth stage, foliage of *Solanum pampasense* seedlings contained 3-14 times the levels of total glycoalkaloids found in the susceptible cultivar, 'Katahdin'. In addition, tubers of potato populations, which were improved in their resistance to potato leafhopper by recurrent selection, contained significantly higher concentrations of α -solanine and α -chaconine (Sanford *et al.*, 1992). In a previous study Sanford *et al.* (1990), demonstrated that the leafhopper infestation declined by 57% after seven generations of selection in *S. tuberosum* populations. The mean concentrations of foliar

solanidine glycosides increased significantly from 40mg/100g fresh weight in the unselected population to 65mg/100g fresh weight in the population developed from seven generations of selection for leafhopper resistance. Recently Sanford *et al.* (1996), showed that potato leafhopper *E. fabae* reared on synthetic diets, had a relatively high mortality (59%) at a 0.09% concentration of α -chaconine.

Resistance to Colorado potato beetle *Leptinotarsa decemlineata* (Say) was first recognised among wild tuber-bearing *Solanum* species of South American origin and in particular in *Solanum demissum*, *S. chacoense* and *Solanum polyadenium* (Greenm.) (Sturckow & Low, 1961; Schreiber, 1957; Kuhn & Low, 1955; Schaper, 1953; Prokoshev *et al.*, 1952). The source of resistance was traced to the major steroid alkaloid of the leaf, a substance called demissine. Although demissine has a similar structure to solanine, it has a repellent effect against Colorado potato beetle which solanine does not. A repellent effect against Colorado potato beetle was observed as well with α -tomatine which has similar chemical structure to demissine. In particular, infiltration of potato leaves with α -tomatine solutions of different concentrations caused 50% reduction of beetle feeding or 100% larval mortality (Schreiber, 1957). Another source of resistance to Colorado potato beetle attack has been identified in some strains of the wild *S. chacoense*. Sinden *et al.* (1980), found that clones of *S. chacoense* with either commersonine or dehydrocommersonine as the major foliar glycoalkaloid were significantly more resistant to Colorado potato beetle than clones with solanine and chaconine. Solanine which is encountered in all *Solanum* species supports the growth of Colorado potato beetle larvae. Growth inhibition in the insect is observed only in concentrations exceeding those usually found in plants, and even in this case the weight gain is approximately 90% that of larvae reared on the alkaloid-free basic diet (Waller & Nowacki, 1978).

Sinden *et al.* (1980), concluded that the types of glycoalkaloids present in the foliage of *S. chacoense* may be as important as the level of total glycoalkaloids in limiting not only the number of Colorado potato beetle larvae and adult insects but also the total damage they cause. Furthermore, these authors suggested that leaf total

glycoalkaloid content was significantly correlated with damage rating but not with number of larvae or adults per plant. Dehydrocommersonine differs from solanine and chaconine only in the size and composition of the sugar group; Tingey (1984), suggested that the number of sugar groups, i.e., a tetrasaccharide vs. a trisaccharide, may be as important if not more important, than the presence of a specific aglycone, or the presence or absence of a particular sugar. In general, leptine I is considered as an extremely effective feeding deterrent while tomatine and demissine are of intermediate activity followed by solanine and chaconine (Tingey, 1984). It is assumed that deterrents like demissine or tomatine act on Colorado potato beetles at the membrane level, possibly interfering with the absorption of the phytosterols of the potato leaf which are required by the beetle for ecdysone synthesis (Harborne, 1993).

Additional studies proved that steroid alkaloids found in *Solanaceae* elicited bursts of electrical activity in galeal and tarsal chemosensilla of adults of *L. decemlineata* (Mitchell & Harrison, 1985). These compounds act independently of any specialised chemoreceptor in *L. decemlineata*, and the association of this insect with solanaceous plants appears not to have led to the evolution of a specific receptor for *Solanum*. In addition Kennedy *et al.* (1985), suggested that the presence of glandular trichomes and other factors involving the glycoalkaloid, α -tomatine, are responsible for the resistance of wild tomato species to the Colorado potato beetle. However Harrison & Mitchell (1988), suggested that tomatine and solanine did not reduce leaf consumption or significantly alter behaviour patterns of newly emerged beetles, including those from populations that normally did not feed on plants containing the compounds. Ghidui *et al.* (1990), showed that the Colorado potato beetle tolerance to pyrethroid toxicity is partially host-plant dependent. The toxicity of permethrin was found to be greater on Colorado potato beetles reared on eggplant compared to those reared on potato. These results demonstrated that a host plant-insecticide resistance interaction possibly exists, suggesting that plant glycoalkaloids are partially responsible for enhancing the beetle's resistance to insecticides. Deahl *et al.* (1991), showed that the larval development rate reduced and the larval mortality

increased, when Colorado potato beetle larvae fed on the foliage of four *S. chacoense* clones grown under high light intensity. The leptine-synthesising clones responded to the increase in light intensity with a 2-4 fold increase in the level of foliar leptines. This resulted to increased resistance to Colorado potato beetle which was attributed to the significant ($P < 0.05$) effect of light on glycoalkaloid synthesis across all clones tested.

Apart from the toxic or feeding deterrent effects of potato glycoalkaloids on the two major potato pests, the potato leafhopper and the Colorado potato beetle, there are a number of studies involving glycoalkaloid effects on other potato pests. In particular Jonasson & Olsson (1994), regarded the total glycoalkaloid content as a key factor of potato tuber resistance to wireworm *Agriotes obscurus* (L.). Avoided feeding sites by the wireworm like this in the outer cortex of the tuber, had low sugar and high total glycoalkaloid content. They concluded (Olsson & Jonasson, 1995) that breeding for high levels of glycoalkaloids in the extreme periphery, but very low levels in the rest of the tuber, will protect tubers from wireworms and yet result in acceptable glycoalkaloid levels for food safety.

Bajaj *et al.* (1989), suggested that glycoalkaloids in association with phenolic compounds in a resistant cultivar of eggplant *Solanum melongena* (L.) may be responsible for resistance to attack by the shoot and fruit borer *Leucinodes orbonalis* (Guen.). By contrast Raman *et al.* (1981), concluded that "primitive" and wild potato accessions with high solanine contents were not resistant to potato tuber moth *Phthorimaea operculella* (Zell.). De Boer & Hanson (1987), studied the feeding response by larvae of the tobacco hornworm *Manduca sexta* (Joh.) to solanaceous allelochemicals including solanine and concluded that solanaceous alkaloids may make a small contribution to food selection by the tobacco hornworm.

Glycoalkaloids contributed to the resistance of potatoes against individuals of the slug *Deroceras reticulatum* (Müller), but they were effective only at levels toxic to man (Cronk *et al.*, 1974). They investigated the resistance of potato cultivars to *D.*

reticulatum damage by feeding raw tuber mash of resistant and susceptible cultivars. The presence of low molecular compounds (glycoalkaloids and phenolics) was essential for expression of resistance. Feeding of high concentrations of purified glycoalkaloids retarded slug growth slightly at concentrations of 67.5mg/100ml of diet and concentrations of 135mg/100ml of diet produced significant weight loss. In addition Forrest & Coxon (1980), found no relationship between the degree of resistance to the potato cyst nematode *Globodera pallida* and glycoalkaloid concentration of the roots or tubers of potato clones derived from *Solanum vernei* (Bitt.) x *S. tuberosum*. Neither did nematode infestation of the roots lead to increased glycoalkaloid concentrations of susceptible or resistant potatoes.

The deterrency and toxicity of glycoalkaloids have also been shown for non potato pests. In particular Chan & Tam (1985), showed that the tomato glycoalkaloid, α -tomatine, exerted an antibiotic effect on larvae of the Mediterranean fruit fly *Ceratitis capitata* (Wied.) Increased concentrations of α -tomatine, resulted in decreased larval survival, lower pupal weights, an extended pupation period and a prolonged period of adult emergence. Tomatine and solanine proved to be the most active feeding deterrents on adults of the flea beetle *Phyllotreta striolata* (F.), a pest of rape in Canada (Meisner & Mitchell, 1984). Eigenbrode & Trumble (1994), found that tomato accessions resistant to the beet armyworm had high concentrations of glycoalkaloids in the fruit tissue.

1.11 Potato glycoalkaloids as resistance factors against the peach potato aphid *Myzus persicae* (Sulzer)

The green peach aphid, *M. persicae* is an agricultural and horticultural pest mainly because it is a major vector of virus diseases of a remarkably wide host range (van Emden *et al.*, 1969). It seldom occurs in very large numbers. On cruciferous crops and potatoes it tends to feed on senescent leaves where its visible effects are unimportant; on other crops, depending to some extent on plant phenology, aphid biotype and the season, it can feed on young tissue-but here too, the immediate

impact of feeding is slight (van Emden *et al.*, 1969). This aphid has a marked preference for highly nutritious plants (van Emden & Bashford, 1969) perhaps because the small quantities of toxicant ingested when aphids feed on tissue rich in nutrients may be quickly detoxified, whereas the larger quantities ingested when feeding on poor-quality hosts may not. Therefore, in selecting for high quality food, polyphagous aphids like *M. persicae* possibly overcome the debilitating effect of some secondary plant metabolites (Dixon, 1985).

Schoonhoven & Derksen-Koppers (1976), in their experiments with artificial diets fed to *M. persicae* showed that the basal diet was apparently preferred to the diet containing solanine in saturated solution; hence α -solanine did not act as a feeding stimulant on *M. persicae*. Although the survival rate on diets containing solanine and tomatine was normal, the numbers of offspring were reduced compared with controls in the first 2-4 days of larviposition. Mackenzie *et al.* (1977), analysed the tissues of 25 tuber-bearing *Solanum* species with different degrees of resistance to *M. persicae* and *E. fabae*, for total glycoalkaloid contents using glycoalkaloids chromatographic analysis. A *S. bulbocastanum* (Dun.) accession, with resistance to *M. persicae*, was found to have very low contents, while a *S. chacoense* accession with susceptibility to *M. persicae*, was amongst those accessions with the highest contents.

Tingey & Sinden (1982), could not find evidence of a causal relationship between foliar or tuber total glycoalkaloid levels and populations of either green peach aphids, leafhoppers, or fleabeetles on 12 accessions of *Solanum berthaultii* (Hawkes) and *S. berthaultii* x *Solanum tarijense* (potato wild relatives) for which both foliar total glycoalkaloid level and resistance were measured. In addition they concluded that glandular trichomes rather than glycoalkaloids provide the major element of resistance in these plants. Mndolwa *et al.* (1984), examined the season-mean colonisation of six potato clones by the peach-potato aphid *Myzus persicae*; he found a weak positive correlation between mean total steroid glycoalkaloid levels and aphid colonisation for all of the six potato clones. This positive correlation suggests an

indirect relationship of peach potato aphid colonisation and foliar total glycoalkaloids to some common determinate such as vine maturity.

1.12 Objectives of the study

The two main characteristics of organic cropping production systems are the absence of synthetic pesticide applications for crop protection and the use of alternative, non-synthetic fertilisers as sources of nitrogen. Spraying or systemic application of insecticides is the normal solution to control increases of pest populations in conventional agriculture. However vast use of insecticides has led to widespread development of resistance by many insect species -among them aphids- and concurrent suppression of the population of natural enemies and predators. In addition it has increased concerns due to the deleterious effects of pesticide residues on human health and the environment. Extensive fertiliser applications not only induced an increase in the nitrate residues in land and water ecosystems, but also increase the nutritive value of the crops and convert the growing plants to ideal host-substrates for herbivorous insect pests.

Although in organic agriculture, several plant protection methods are based on increasing the numbers of natural enemies combined with applications of biopesticides, it is believed that organically grown plants are totally unprotected and as a result they suffer more stress, induced from insect infestations. By contrast it would be expected that conventionally grown plants are subjected to lower or no stress effects, because synthetic pesticide applications either reduce to a minimum level, or completely exclude pests of the agricultural ecosystem. Ames *et al.* (1990), postulated that plants which are stressed or damaged through attacks by or in competition with pests may produce higher levels of natural pesticides than unstressed plants. Hence it would be supposed that organically grown plants are producing elevated amounts of natural pesticides because they suffer more stress due to higher insect infestations. However this increase of natural pesticides in the plant tissues could mean that a crop could develop to a degree a self-defence system

protecting it from further pest attacks.

The aim of this project was to investigate the hypothesis that aphid-infested potato plants produce elevated amounts of glycoalkaloids, compared to the amounts produced by non-infested plants. A potential aphid-induced increase in the foliar glycoalkaloid content could further support the idea that the aphid-infested potato plants are able to develop a natural defence system. This could imply that in conventional and organic production, the natural defence mechanisms existing, should be taken into consideration when a crop protection plan is designed. The common cultivated potato *Solanum tuberosum* L. proves to be an excellent food crop for studying the occurrence of natural toxins. As a member of the Solanaceae family, potato plants also readily synthesise natural pesticidal toxins known as glycoalkaloids. The inhibitory effects of glycoalkaloids on both fungal pathogens and insect pests of the potato indicate that their evolutionary significance is most likely that of natural pesticides. However the role of glycoalkaloids as natural toxins against the aphid *M. persicae* has not been clearly investigated. For this reason, the effects of glycoalkaloids on the lifespan parameters of *M. persicae* were decided to be examined first using artificial diets. A potential adverse effect of potato glycoalkaloids on the aphid *M. persicae*, could possibly implicate these substances as being a part of a complex defence mechanism against aphids. *M. persicae* was preferred to other potato pests because of its importance as a major pest of potato crop. Experimentation with a peach potato aphid clone infesting potato plants could further reduce variation in the results.

Alteration of secondary metabolism in a plant can change the plant responses to biotic stress (pathogens, insects, nematodes and other plants) and to stress imposed by the physical environment. An additional stress-inducing factor is regarded to be the deficiency of nutrients and more particularly that of nitrogen. In organic production systems the amounts of available nitrogen to the crop are limited while in conventional systems the rule is the nitrogen sufficiency. Since glycoalkaloids are nitrogenous compounds it is expected that modification of the nitrogen availability to

potato plants, could alter the glycoalkaloid concentrations in the potato foliage and as a consequence possibly affect indirectly the aphid individual performance as well as the rates of aphid infestation. In addition there is little known of the effects of insecticides on the secondary metabolism of the plants. For this reason, the effects of nitrogen availability and a systemic pesticide (aldicarb) systemic application on the foliar glycoalkaloid synthesis were examined in potato plants grown in a semi-hydroponic system. Furthermore, the effects of aphid infestation as a stress-inducing factor on the foliar glycoalkaloid production were examined in a semi-hydroponic culture system and in a field experiment. To summarise the objectives of the study:

1. To study the effects of potato glycoalkaloids on the peach potato aphid *M. persicae* establishing links between these naturally occurring secondary metabolites and the life parameters of this aphid when reared in artificial diets.
2. To examine the effect of nitrogen availability as well as that of systemic pesticide application, on the glycoalkaloid production of potato plants grown in controlled environment.
3. To draw conclusions about the potential aphid stress-induced alteration in glycoalkaloid production of potato plants, exposed either to different quantities or different types of nitrogen fertilisation, when grown in controlled environment and field conditions respectively.

Chapter 2

Effects of potato glycoalkaloids on life parameters of the aphid *Myzus persicae*

2.1 Introduction

Although glycoalkaloids are considered to contribute to plant defence mechanisms possessing insecticidal properties (Sanford *et al.*, 1996; Tingey, 1984), their role as potato plant allelochemicals in relation to the polyphagous peach-potato aphid *M. persicae* (Sulzer) (Homoptera: Aphididae), has been only partially investigated. In particular there is only one study examining the effects of α -solanine on *M. persicae* when offered in artificial diets in saturated solution. In food selection experiments using artificial diets Schoonhoven & Derksen-Koppers (1976), showed that α -solanine did not enhance diet acceptability by *M. persicae* when incorporated to the basal diet, therefore this substance did not act as a feeding stimulant. Moreover, in a no-choice situation, nymphs of *M. persicae* fed on an artificial diet containing α -solanine, showed normal survival after 2 and 4 days.

Little information exists in regard to the possible effects of these substances on the various life parameters of this aphid. The aim of this study was to investigate the lifespan effects of the two major potato glycoalkaloids (α -solanine and α -chaconine) on the performance of *M. persicae* reared on artificial diets. The artificial diet method was used because in this way the possible effects of glycoalkaloids on the aphid performance could be clearly observed, keeping constant other nutrients that could also affect aphid performance. The performance of the aphids was measured in terms of fecundity, longevity, diet acceptability, nymphal development and population growth rate.

2.2 Materials and Methods

Three experiments were conducted in the plant growth unit of SAC. In the preliminary experiment (pH exp.) apterous adult aphids were placed to reproduce in acidic and neutral artificial diets. In the following two experiments, apterous adult aphids (exp. 1) and nymphs (exp. 2) were reared in glycoalkaloid (GA) containing artificial diets.

2.2.1 Host plant culture for the rearing of the aphid colony

The aphids used in both artificial diet and potato experiments (chapters 4&5), derived from an aphid colony established on Chinese cabbage (*Brassica pekinensis* (Lour) Brassicaceae cv. Kasumi) leaves. Chinese cabbage was a convenient source of glycoalkaloid-free leaves. Therefore the aphids which were used in both artificial diet and plant experiments had no previous glycoalkaloid experience. Chinese cabbage plants were cultivated in a plant growth cabinet (Fisons model 600G3/THTL) at 25°C, 50% RH, and long day (16:8) conditions. The plants were grown in plastic pots (diameter: 15cm; depth: 14cm) which were filled with 1:1 mix (by volume) of horticultural grade perlite and Irish moss peat; the pots stood in black plastic trays (22cm x 16.5cm x 4.5cm). Liquid plant food (Levington all purpose) was added with watering (0.5ml fertiliser/l water) to support vegetative growth and continuous supply of leaves. Once every two weeks the plant foliage was immersed in a soft soap solution to prevent plant infestation by other insects or colonisation by invading aphids. Regular sowings every three months constantly provided plants producing leaves at the appropriate growth stage for aphid colonisation. The leaves necessary for the aphid colony were excised using a sharp knife and placed in small (diameter: 3cm; depth: 7cm) clear polypropylene cylinder bottles with the freshly cut ends immersed in water. Each leaf was then caged in a clear conical (diameter: 15cm; depth: 14cm) polypropylene propagator top having two 5cm-diameter holes covered by very fine nylon mesh. For additional protection vaseline was applied to the rims of each propagator top and the whole system was placed in a plastic tray (six leaves per tray). In this way, adequate light, ventilation and “aphid proofing” were achieved.

2.2.2 Rearing procedure

A clone lineage originating from one isolated individual female peach-potato aphid *M. persicae* was established. In particular, one individual apterous female adult was collected on oilseed rape on 8 June 1995 in Lothian Region Scotland. The aphid was placed to reproduce on an excised Chinese cabbage leaf in a climatic room at 25°C, 50% RH, and long day (16:8) conditions for 12 hours and then discarded. These

temperate environmental conditions were inhibiting the production of aphid sexual morphs. The offspring produced developed to adults after 6.5 days and then were placed to reproduce on Chinese cabbage leaves for 12 hours. By applying the “method of successive generations” (Harrington, 1945), a control over the number of aphids produced was achieved. Only the first-born progeny of each generation were kept: the adults were discarded as soon as they had produced the requisite number of progeny on the excised leaf. Thus the development of all individuals in a clone was effectively synchronised. According to Blackman (1988), this method is the best way to rear aphids for experimental work, where it is important to start with a batch of individuals which are all at the same developmental stage.

2.2.3 Artificial diet formulation and method of use

The diet used in this study is that of Dadd & Mittler (1966). The diet composition is presented in Table 2.1. The purity of the water used as solvent for the nutrients in the artificial diet is of great importance. According to Adams & van Emden (1972), bad water quality may lead to poor success with artificial diets. For this reason the water used for dissolving the diet components was Rathburn high purity HPLC grade water, filtered through a 47 mm diameter, 0.45 µm pore size Whatman® sterile membrane filter to remove impurities, sterilised by autoclaving (15min, 122°C, 1.15bar) in Schott Duran 1000ml bottles and stored until use.

Preparation of the basic stock solutions: three different stock solutions of amino acids, vitamins and salts were prepared. The amino acid stock solution was prepared by weighing and diluting the amino acids required by the recipe in water in a beaker placed on a hotplate stirrer at 45°C. After dilution the final volume was adjusted to 500ml. The vitamin and mineral stock solutions were prepared by weighing and diluting the vitamins and salts required in water at 25°C. The final volumes of the vitamin and mineral stock solutions were adjusted to 200ml and 100ml respectively. The stock solutions prepared were used for the formulation of the glycoalkaloid - containing and the pure (non-glycoalkaloid - containing) artificial diets presented to the aphids. Two stock solution artificial diets were prepared. In the first no

glycoalkaloids were added, while in the second the glycoalkaloids were added at a concentration of 320mg/100ml.

Table 2.1. Composition of artificial diet

L-amino acids	Weight (mg/100 ml diet)	Vitamins, Minerals and Sucrose	Weight (mg/100 ml diet)
Alanine	100	Ascorbic acid	100.0
Arginine	270	Thiamin HCl	2.8
Asparagine	550	Riboflavin	0.5
Aspartic acid	140	Nicotinic acid	10.0
Cysteine HCl	40	Pyridoxin	2.5
Glutamic acid	140	Folic acid	0.5
Glutamine	150	Ca Pantothenate	5.0
Glycine	80		
Histidine	80	Inositol	50.0
Isoleucine	80	Choline chloride	50.0
Leucine	80	Biotin	0.1
Lysine HCl	120	KH ₂ PO ₄	500.0
Methionine	40	MgCl ₂ ·6H ₂ O	200.0
Phenylalanine	40		
Proline	80	Fe Sequestrene	1.5
Serine	80	Zn Sequestrene	0.8
Threonine	140	Mn Sequestrene	0.8
Tryptophane	80	Cu Sequestrene	0.4
Tyrosine	40		
Valine	80	Sucrose	15,000
Total amino acids	2410		

Preparation of the control artificial diet: this was prepared by mixing 250 ml of the amino acid stock solution with 100 ml of vitamin and 50 ml of mineral stock solutions with 75g of sucrose and water up to 500 ml (final volume). The initial pH of the non GA-containing artificial diet was 4.6, but this was adjusted to 6.0 by adding small amounts of 1N KOH solution to the diet solution before the final volume adjustment to 500ml.

Preparation of the diets used in the preliminary experiment (pH exp.):

Three non GA-containing artificial diet solutions were prepared. Each one was prepared by mixing 12.5 ml of the amino acid stock solution with 5 ml of vitamin and 2.5 ml of mineral stock solutions and adding 3.75 g of sucrose. The pH values of these three different solutions were adjusted to non acidic (NA, pH=7.0), medium acidic (MA, pH=6.0) and highly acidic (HA, pH=4.6) respectively, by adding small

amounts of 0.1N KOH solution to the prepared solution. The final volume was adjusted to 25 ml using high purity HPLC water.

Preparation of GA-containing diets: this was prepared by mixing 12.5 ml of the amino acid stock solution with 5 ml of vitamin and 2.5 ml of mineral stock solutions with 3.75 g of sucrose, 32 mg of α -solanine, 48 mg of α -chaconine and water up to 25 ml (final volume). In this way, the initial concentration of glycoalkaloids in the diet was 320mg/100ml. The α -solanine to α -chaconine ratio (1:1.5) in this solution was similar to the ratio observed in potato leaves (Guseva *et al.*, 1960).

The initial pH of the GA-containing artificial diet was 5.5, but this was adjusted to 6.0 by adding small amounts of 0.1 N KOH solution, in the diet solution before the final volume adjustment to 25 ml. Although the original recipe suggested that the pH of an artificial diet should be 7.0, the modification of the pH proved to be essential, owing to the poor solubility of glycoalkaloids in water solutions with pH higher than 5.6 (Mitchell & Harrison, 1985). In the glycoalkaloid containing artificial diet, these substances were precipitated at a pH higher than 6.2. The glycoalkaloids α -solanine and α -chaconine which were used in the experiments comprise 95% of the total potato foliar glycoalkaloids (Jadhav *et al.*, 1981). They were obtained (purity approx. 95%) from Sigma-Aldrich Co Ltd, Fancy Rd, Poole, Dorset, BH17 7NH England. The different artificial diet treatments were derived by double diluting equal amounts (10 ml) of both solutions (GA-containing and non GA-containing) in volumetric flasks. In particular, the treatments were T_0 , T_{10} , T_{20} , T_{40} , T_{80} , T_{160} , representing 0 (control), 10, 20, 40, 80, and 160mg of GA/100ml of diet respectively. The glycoalkaloid concentration in the potato leaves ranges between 30-160 mg/100g FW (Dao & Friedman, 1996).

2.2.4 Osmolality measurements

The osmolality values of the pure (0 mg of GA/100ml of diet) and the GA-containing (160 mg of GA/100ml of diet) artificial diets were measured using a Wescor 5100C vapour pressure osmometer and found not to be significantly different (822.25

mmol/kg, and 822.75mmol/kg, respectively $t=0.47$ for 5df, $P>0.05$).

2.2.5 Procedures and assessment of aphid performance

The preliminary adult and nymph experiments were conducted in a climatic room at 25°C, 50% RH, and long day (16:8) conditions.

Preliminary experiment (pH exp)

Standardised pre-reproductive adult aphids obtained from the Chinese cabbage leaves, were placed to feed and reproduce in cages containing artificial diets with three different pH values. The aphids were caged (4 replication-cages per artificial diet treatment, 5 apterous adult aphids per cage) and fed with the artificial diet through the Parafilm® membrane (Mittler & Dadd, 1964). Individual fecundity (number of new born nymphs/apterous adult) and mortality (number of dead adult aphids/ cage) were measured with newborn nymphs and dead adults counted and discarded every 24 hours. Due to decomposition of the diet, the living adult aphids were transferred (every 48 hours) to cages with fresh diet, for a total period of 30 days (entire aphid lifespan). Diet uptake for each diet-containing cage was calculated from the difference in the cage weight recorded before and after aphid feeding for the first 48 hours of the experiment. Weight loss for each cage due to water evaporation was considered as equal for all artificial diet treatments. From a statistical viewpoint, the experiment was totally randomised with one treatment applied at three levels (NA, pH=7.0; MA, pH=6.0; HA, pH=4.6). Data were analysed separately at 4-8 different time points for each variable. The overall effect of the pH treatments was measured by a variance ratio test using MINITAB® (vs.11.1) and the differences between means measured by t-test.

Adult experiment (exp. 1)

The adult aphid population used originated from nymphs born during 12 hours, on Chinese cabbage leaves. After their third ecdysis the standardised 4th instar nymphs, were left to starve for 12 hours and were then transferred (using a moistened fine sable-hair brush) to glass cylinder cages for adaptation to the artificial diet substrate

and caging conditions. The cages contained pure artificial diet (not containing glycoalkaloids) as a thin layer between two pieces of stretched Parafilm® membrane (Mittler & Dadd, 1964).

The adults which followed the final ecdysis were transferred to the GA-containing artificial diet cages (4 replication-cages per artificial diet treatment, 5 apterous adult aphids per cage) to feed and reproduce in the controlled climate room. Individual fecundity (number of new born nymphs/apterous adult) and mortality (number of dead adult aphids/ cage) were measured as in the previous experiment, with newborn nymphs and dead adults counted and discarded every 24 hours. The living adult aphids were transferred (every 48 hours) to cages with fresh diet, for a total period of 34 days (entire aphid lifespan). Diet uptake for each diet-containing cage was calculated from the difference in the cage weight recorded before and after aphid feeding for the first 48 hours of the experiment.

Nymph experiment (exp. 2)

The nymph population used was born from female adults which were transferred from Chinese cabbage leaves to cages containing pure artificial diet where they remained for 6 hours to reproduce. The standardised nymphs remained on the pure artificial diet for 12 hours to gain weight and then were weighed using a Sartorius microbalance (model: Ultramicro) and transferred to the glycoalkaloid diet cages (4 replication-cages per artificial diet treatment, 15 nymphs per cage) to feed and develop in the same controlled climate room. The nymphs were reweighed and transferred to cages with fresh glycoalkaloid diet two more times before becoming adults. After their final ecdysis and before starting to reproduce they were reweighed for the last time (as pre-reproductive adults) and five apterae were placed again on the diets to reproduce. From the weight measurements, the weight increase as well as the mean relative growth rate (MRGR) of the living nymphs in each cage were calculated. The MRGR was calculated by the formula given by van Emden (1969):

$$\text{MRGR} = \frac{[\ln(\text{final weight}) - \ln(\text{initial weight})]}{[\text{growth period (days)}]}$$

The nymphs generally (with the exceptions of T_{80} and T_{160}) took about 6.5 days before they themselves reproduced, and the next generation of nymphs they produced were counted, weighed and removed daily. In total the aphids fed on the diets 13 days on T_0 - T_{40} and 16 days on T_{80} . The Intrinsic Rate of Natural Increase (r_m) was then calculated for each cage of aphids according to the formula given by Wyatt & White (1977):

$$r_m = 0.74((\ln M_d)/d)$$

where d is the pre-reproductive time in days (from birth to first reproduction), and M_d is the number of progeny produced in the ensuing period of length d .

From a statistical viewpoint, the experiments were totally randomised with one treatment applied at six levels (T_0 - T_{160}). Data were analysed separately at 4-6 different time points for each variable. The overall effect of the treatments was measured by a variance ratio test using MINITAB® (vs.11.1) and the differences between means measured by t-test.

2.3 Results

2.3.1 Preliminary experiment (pH exp.)

Adult aphids were transferred from the Chinese cabbage to the cages and subsequently settled on to the artificial diet 3-4 hours later. This delay in settling of the aphids might be due to a difficulty of adaptation in caging conditions, or a result of aphid saturation after feeding on the Chinese cabbage leaves. Figure 2.1 illustrates the reproductive performance of *M. persicae* adults fed on non acidic (NA pH=7.0 - control), medium acidic (MA pH=6.0) and highly acidic (HA pH=4.6) artificial diets. The reproductive performance is expressed as the total number of nymphs born per adult for each of the first five days of the experiment. No significant differences ($P>0.05$) were found in the reproductive performance between aphids fed on the control (NA) and on the medium acidic (MA) diets for any of the five days observed. The difference between MA (pH=6.0), NA (pH=7.0) and the HA (pH=4.6) diets was significant ($P<0.01$) during the first two days of the experiment and became highly

significant ($P < 0.001$) during the next two.

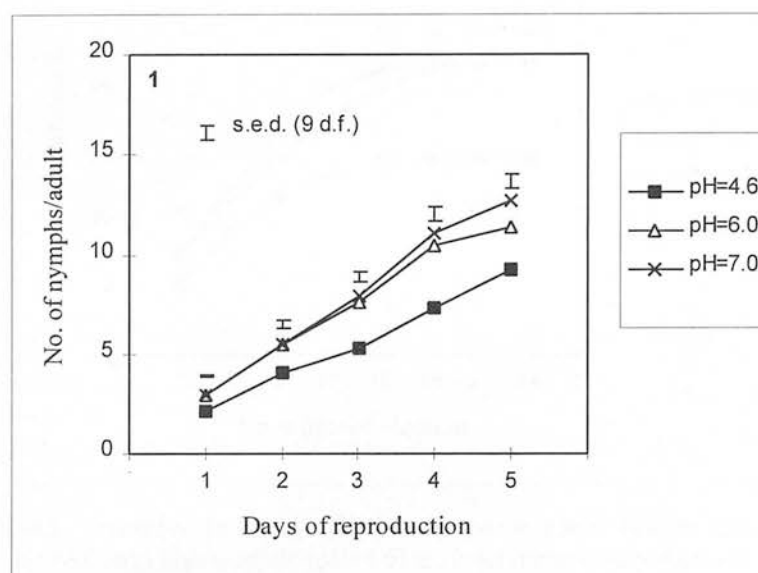


Figure 2.1. Mean cumulative fecundity of *Myzus persicae* adults fed on non-acidic (pH=7.0), medium acidic (pH=6.0) and highly acidic (pH=4.6) artificial diets for the first five days of their reproductive life.

At the end of the period (5th day) aphids fed on the HA (pH=4.6) diet produced a significantly lower number of nymphs compared to those fed on the MA (pH=6.0) diet ($P < 0.05$) and to those fed in the NA (pH=7.0) diet ($P < 0.01$).

Figure 2.2 shows the reproductive performance of the aphids as this is expressed in the total number of nymphs born per adult in three day intervals for the entire reproductive period of the adults. The duration of the reproductive period (date on which the last nymph was born) was the same for the NA (pH=7.0) and the HA (pH=4.6) diet and lasted 18 days. This period was prolonged to 24 days for the aphids fed on the MA (pH=6.0) diet. No significant ($P > 0.05$) difference was observed between the total number of nymphs born in MA (pH=6.0) and NA (pH=7.0) diets. By contrast aphids fed on the HA (pH=4.6) diet produced significantly ($P < 0.01$) lower number of nymphs to those produced on the MA (pH=6.0) and the NA (pH=7.0) diets.

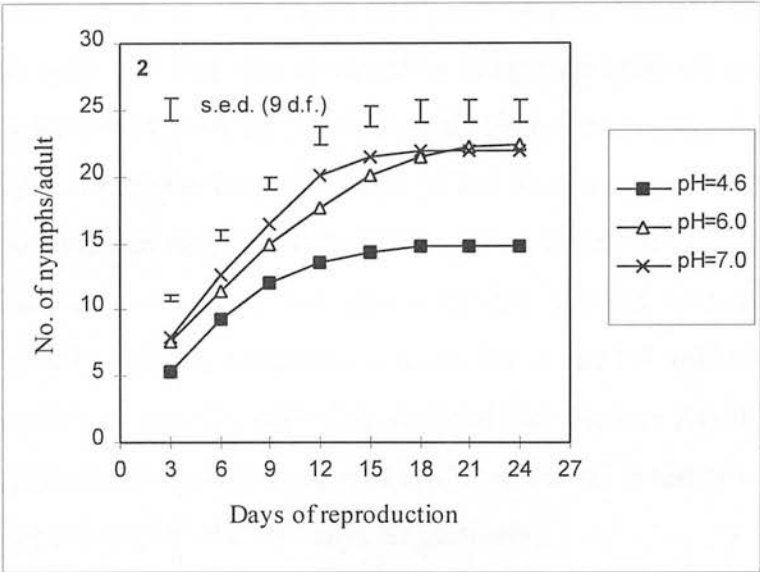


Figure 2.2. Mean cumulative fecundity of *Myzus persicae* adults fed on non-acidic (pH=7.0), medium acidic (pH=6.0) and highly acidic (pH=4.6) artificial diets at eight different time intervals for the entire reproductive period (from 3 to 24 days).

Figure 2.3 shows the mean cumulative mortality of the aphids fed on the same artificial diets. Aphids fed on the MA (pH=6.0) diet showed normal longevity to those fed on the NA (pH=7.0) diet.

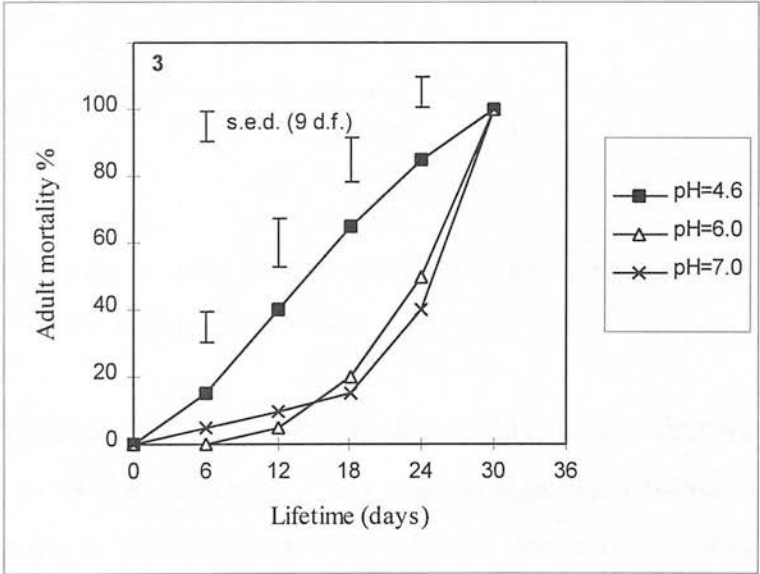


Figure 2.3. Mortality of *Myzus persicae* adults fed on artificial diets of different acidity (pH= 4.6-7.0), at five different time intervals across lifespan (from 6 to 30 days).

In particular no significant differences ($P>0.05$) were observed in the number of dead aphids on the NA (pH=7.0) and the MA (pH=6.0) artificial diets for the total period

of the experiment. A higher (although non significant) mortality of the adult aphids fed on the MA (pH=6.0) diet was observed in the period between the 12th and 18th days. Aphids fed on the HA (pH=4.6) diet showed significantly ($P<0.05$) higher mortality compared to those fed on the MA (pH=6.0) diet on the 12th and 18th days. This difference remained significant ($P<0.01$) on the 24th day of the experiment. In a similar way, aphids fed on the HA (pH=4.6) diet showed higher although not significant ($P>0.05$) mortality compared to those fed on the NA (pH=7.0) diet on the 12th day. Mortality in this HA (pH=4.6) artificial diet became significant ($P<0.01$) and highly significant ($P<0.001$) compared to that observed in the NA (pH=7.0) diet in the next two periods (18th and 24th days respectively).

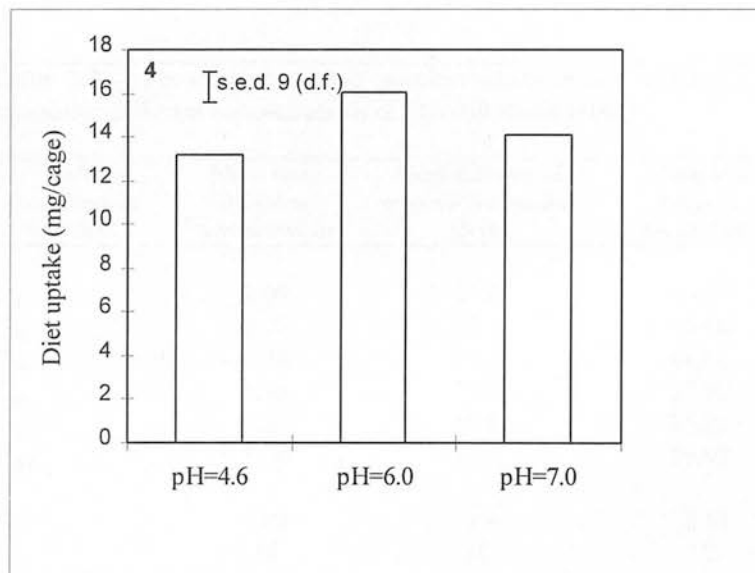


Figure 2.4. Diet uptake by *Myzus persicae* adults and offspring during 48 hours of feeding on artificial diets of different acidity (pH= 4.6-7.0).

Figure 2.4 illustrates the diet uptake by the aphids for the first 48 hours of the experiment expressed in mg/cage. There was no significant ($P>0.05$) difference of diet uptake for any of the different artificial diet pH treatments. Diet uptake appeared to be higher in the MA (pH=6.0) diet.

2.3.2 Adult experiment

Table 2.2 presents the fecundity data from the adult aphid experiment. There was a

highly significant ($P < 0.001$) effect of glycoalkaloid concentration on the mean daily offspring of the aphids due to the decreased mean number of new born nymphs observed in T_{160} treatment. Aphids fed on T_{80} treatment produced significantly fewer daily offspring compared to that of the control ($P < 0.05$), T_{10} and T_{20} ($P < 0.001$) treatments. Although the mean daily number of nymphs produced in low glycoalkaloid concentrations (T_{10} , T_{20}) was significantly higher ($P < 0.05$) compared to that of the control, no significant ($P > 0.05$) differences were found between the total number of nymphs produced in control and T_{10} - T_{20} treatments. In addition, no significant ($P > 0.05$) differences were found in the mean daily offspring produced between control and T_{40} , while the total number of nymphs produced in this treatment was significantly ($P < 0.01$) lower compared to that of the control.

Table 2.2. Fecundity of *Myzus persicae* adults fed on artificial diets containing different concentrations of glycoalkaloids (GAs)

GA concentration mg/100ml	Mean daily offspring (nymphs/adult)	Mean duration of reproductive period (days)	Mean total progeny (nymphs/adult)
T_0	2.09	21.8	45.57
T_{10}	2.28	21.3	48.60
T_{20}	2.30	19.3	44.40
T_{40}	1.93	19.5	37.62
T_{80}	1.85	19.0	35.22
T_{160}	1.50	13.3	19.92
SED	0.09	1.4	2.41
DF	18	18	18
P	***	***	***
SED: standard error of difference between means, DF: degrees of freedom *** $P < 0.001$			

The duration of the reproductive period appeared to be similar ($P > 0.05$) among control and glycoalkaloid treatments with the exception of the significantly ($P < 0.001$) reduced reproductive period observed in the highest (T_{160}) glycoalkaloid concentration. The aphid mean total progeny was significantly ($P < 0.001$) affected by the glycoalkaloid concentration in the diet. In particular, highly significant ($P < 0.001$) differences were found between the total number of nymphs produced in control and T_{80} - T_{160} treatments. Adult aphids fed on pure artificial diet produced 1.3 and 2.3 times more nymphs than those produced from aphids fed on T_{80} and T_{160}

treatments, respectively. The total number of nymphs produced by aphids feeding on the two medium concentration (T_{40} , T_{80}) glycoalkaloid treatments appeared to be similar ($P>0.05$), but significantly ($P<0.001$) higher compared to the nymph number produced in T_{160} treatment.

Figure 2.5 illustrates the reproductive performance of the aphids, expressed as total number of nymphs born per adult during the first five days of reproductive life. Over this period, there was a highly significant ($P<0.001$) effect of glycoalkaloid concentration on cumulative fecundity. This was largely due to the poorer fecundity observed on T_{80} and T_{160} treatments in comparison to that observed in control. In particular, during the first two days of reproduction a highly significant ($P<0.001$) difference was found between low-medium (T_0 , T_{10} , T_{20} , T_{40} , T_{80}) and high (T_{160}) treatments. No significant ($P>0.05$) differences were found between low (T_0 , T_{10} , T_{20}) and medium (T_{40} , T_{80}) glycoalkaloid treatments, while the number of offspring produced in T_{40} during the first day was significantly ($P<0.05$) higher than that produced in T_{80} .

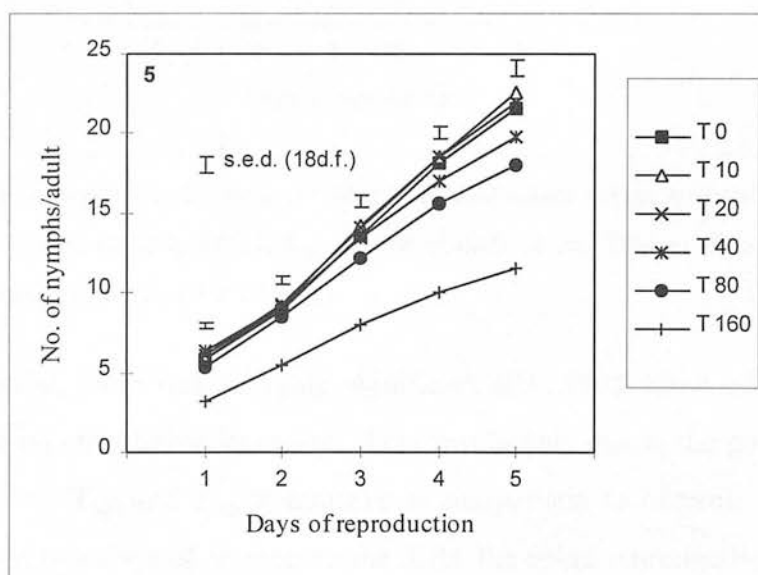


Figure 2.5. Mean cumulative fecundity of *Myzus persicae* adults fed on artificial diets containing different glycoalkaloid (GA) levels (T_0 - T_{160} mg/100ml diet), for the first five days of reproductive life.

On the third day of the experiment, the effects of the glycoalkaloids on aphid performance were significant with T_{80} ($P<0.05$) and T_{160} ($P<0.001$) treatments. The

total number of nymphs produced on T_{80} by the fifth day was significantly lower compared to that in control ($P<0.01$), T_{10} and T_{20} ($P<0.001$) treatments. The highest fecundity was observed on T_{10} treatment, but this was not significantly different ($P>0.05$) from the fecundity observed on control and T_{20} treatment.

Figure 2.6 illustrates the reproductive performance of the aphids as this is expressed in the total number of nymphs born per adult in four day intervals for the entire reproductive period of the adult.

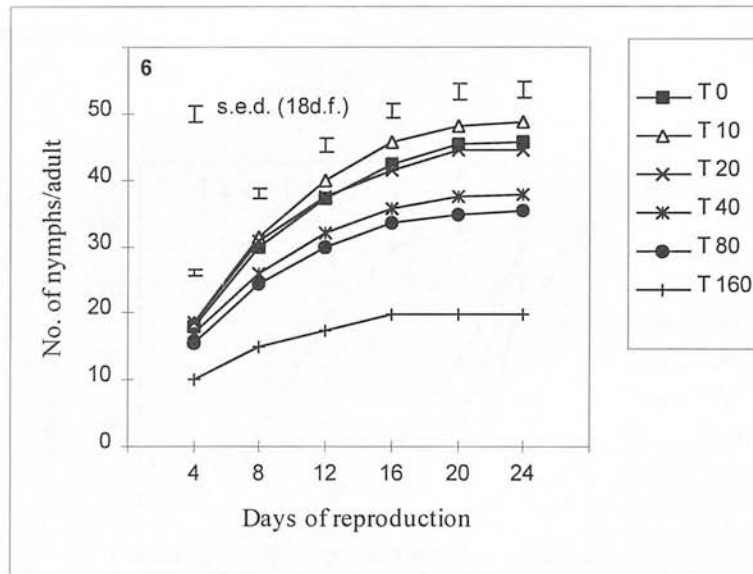


Figure 2.6. Mean cumulative fecundity of *Myzus persicae* adults fed on artificial diets containing different glycoalkaloid (GA) levels (T_0 - T_{160} mg/100ml diet), at six different time intervals for the entire reproductive period (from 4 to 24 days).

Over this period, there was a highly significant ($P<0.001$) effect of glycoalkaloid concentration on cumulative fecundity. This was largely due to the poorer fecundity observed on T_{40} , T_{80} , and T_{160} treatments in comparison to control. In particular, during the first four days of feeding on the diets, the aphid reproductive performance was significantly lower in T_{80} ($P<0.01$) and T_{160} ($P<0.001$) treatments compared to control, while no significant ($P>0.05$) differences were observed in fecundity between control and T_{40} treatments. In addition, highly significant ($P<0.001$) differences were observed between medium (T_{40} - T_{80}) and high (T_{160}) treatments. From the 8th to the 24th day of the experiment, highly significant ($P<0.001$)

differences in aphid reproductive performance were found between low (T_0 , T_{10} , T_{20}), medium (T_{40} , T_{80}) and high (T_{160}) concentrations of glycoalkaloids. The highest fecundity was observed on T_{10} treatment but this was not significantly different ($P>0.05$) from the fecundity observed on control and T_{20} treatments.

The effects of glycoalkaloids on adult aphid longevity are shown in Figure 2.7. There were no significant ($P>0.05$) effects of glycoalkaloids on longevity during the first five days of the experiment, while after ten days of feeding the effects of glycoalkaloids were highly significant ($P<0.001$) in the highest (T_{160}) glycoalkaloid concentration treatment.

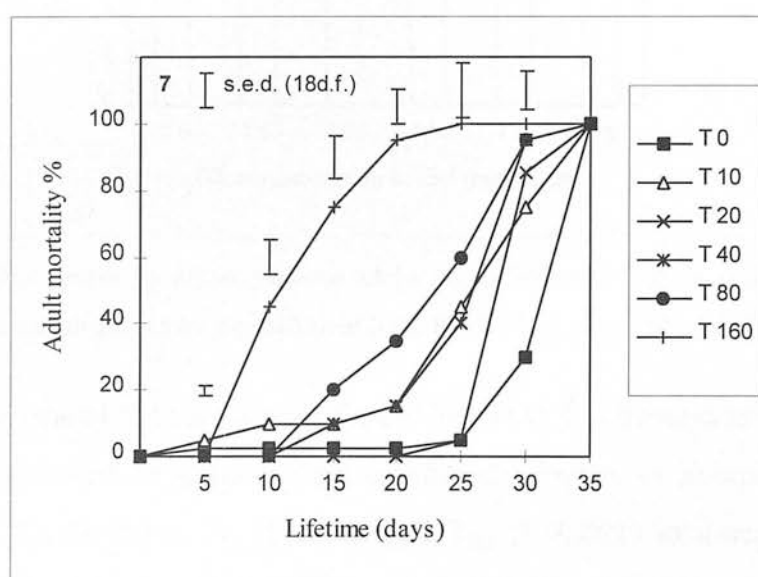


Figure 2.7. Mortality of *Myzus persicae* adults fed on artificial diets containing different glycoalkaloid (GA) levels (T_0 - T_{160} mg/100ml diet), at seven different time intervals across lifespan (from 5 to 35 days).

Most of the aphids did not settle on the diet containing the highest glycoalkaloid (T_{160}) treatment. No significant ($P>0.05$) differences were observed in any of the treatments -with exception of T_{160} in which adult mortality was significantly $P<0.001$ higher compared to all other treatments- on the 15th day of the experiment. On the 25th day of the experiment, the aphid mortality on T_{10} was similar ($P>0.05$) to that observed on T_{40} and T_{80} treatments and significantly ($P<0.05$) higher than that observed on control and T_{20} treatments.

The effects of glycoalkaloids on the diet uptake of *M. persicae* for the first 48 hours of the experiment are presented in Figure 2.8. There was a highly significant ($P<0.001$) effect of glycoalkaloids on diet uptake.

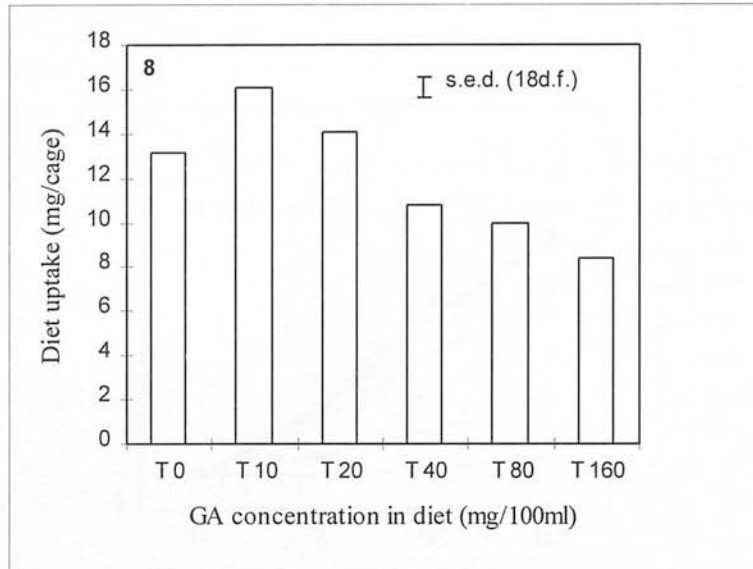


Figure 2.8. Diet uptake by *Myzus persicae* adults and offspring during 48 hours of feeding on artificial diets containing different glycoalkaloid (GA) levels (T₀-T₁₆₀ mg/100ml diet).

The uptake appeared to be highest in T₁₀ and lowest in T₁₆₀ treatments. In particular, the uptake observed in control was significantly higher in comparison to that observed in T₄₀ ($P<0.05$), T₈₀ ($P<0.01$) and T₁₆₀ ($P<0.001$) treatments. The diet uptake was significantly higher in T₁₀ than in control ($P<0.01$) and T₂₀ ($P<0.05$) treatments.

2.3.3 Nymph experiment

The standardised nymphs remained on the pure (GA-free) artificial diet for 12 hours to gain weight before transfer to the GA-containing artificial diets. Figure 2.9 shows the weight increase of *M. persicae* nymphs fed on the GA-containing artificial diets from 12 (1st instar nymphs) until 156 (pre-reproductive adults) hours of age. Medium (T₄₀, T₈₀) and high (T₁₆₀) concentrations of glycoalkaloids reduced the weight gain of nymphs and led to a substantial (T₈₀) or infinite delay (T₁₆₀) of maturation.

Since 1st instar nymphs were feeding on the pure (GA-free) artificial diet for the first 12 hours of their life no significant ($P>0.05$) differences were observed on their weight gain for that period. The weight of 72-hours old nymphs fed for 60 hours on T_0 , T_{10} , T_{20} and T_{40} treatments, was significantly ($P<0.001$) higher compared to T_{80} and T_{160} treatments.

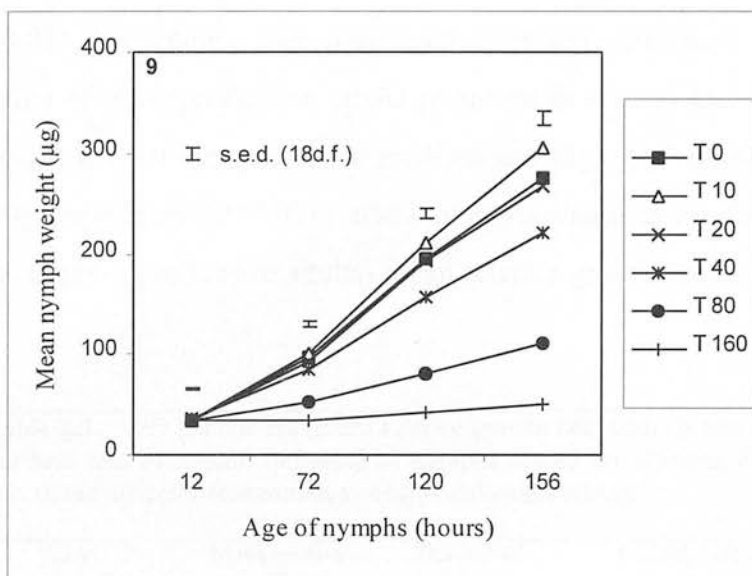


Figure 2.9. Weight increase of *Myzus persicae* nymphs reared on artificial diets containing different glycoalkaloid levels (T_0 - T_{160} mg/100ml diet), at 12, 72, 120 and 156 hours of feeding.

During the same period, the weight of nymphs fed on T_{40} treatment was significantly lower compared to control ($P<0.05$) and T_{10} ($P<0.01$) treatments. No significant ($P>0.05$) differences were observed between aphids fed on T_{40} and T_{20} treatments as well as between T_{10} , T_{20} and T_0 treatments. In addition aphids fed on T_{80} treatment were significantly ($P<0.01$) heavier compared to those fed on T_{160} treatment.

The weight difference between aphids fed on T_{40} and those fed on control (T_0), T_{10} and T_{20} treatments became highly significant ($P<0.001$) at the next weight measurement (120 hours of feeding). As in the previous period there was a highly significant effect ($P<0.001$) of glycoalkaloids on the weight gain between nymphs fed on T_{80} and T_{160} and nymphs fed on T_0 , T_{10} , T_{20} and T_{40} treatments. No significant ($P>0.05$) differences were observed between aphids fed on T_0 , T_{10} and T_{20} treatments, while aphids fed on T_{80} treatment were significantly ($P<0.001$) heavier compared to

those fed on T₁₆₀ treatment.

No adults were observed in T₈₀ and T₁₆₀ treatments after 156 hours of feeding, while the adults observed in T₄₀ treatment were significantly lighter than those observed in control, T₁₀ ($P<0.001$) and T₂₀ ($P<0.01$) treatments. Pre-reproductive adults fed on T₁₀ treatment were significantly heavier compared to those fed on control ($P<0.05$) and T₂₀ ($P<0.01$) treatments. No significant ($P>0.05$) difference was observed between weights of pre-reproductive adults produced in control and T₂₀ treatments. Decreased weight gain of nymphs in the medium and high glycoalkaloid treatments led to a highly significant ($P<0.001$) effect of glycoalkaloids on their overall (1st instar nymphs to pre-reproductive adults) mean relative growth rate (MRGR) (Table 2.3).

Table 2.3. Life parameters (mean relative growth rate, ecdysis rate and intrinsic rate of natural increase) of nymphs reared on artificial diets containing different concentrations of glycoalkaloids (GAs).

GA concentration mg/100ml	Mean relative growth rate μg/μg day	Number of nymphs ecdysed/day	Intrinsic rate of natural increase (r_m)
T ₀	0.37	8.34	0.310
T ₁₀	0.39	8.77	0.313
T ₂₀	0.37	8.50	0.313
T ₄₀	0.33	7.50	0.283
T ₈₀	0.21	5.46	0.204
T ₁₆₀	0.07	2.11	0.000
SED	0.01	0.37	0.008
DF	18	18	18
P	***	***	***

SED: standard error of difference between means, DF: degrees of freedom
*** $P<0.001$

This was mainly due to poor development of nymphs observed in T₈₀ and T₁₆₀ treatments. Although pre-reproductive adults fed on T₁₀ treatment were significantly ($P<0.05$) heavier compared to those fed on control, no significant ($P>0.05$) differences were observed in the MRGRs between these two treatments. No significant ($P>0.05$) differences were observed in MRGRs of aphids grown on T₀ - T₂₀ and T₁₀ - T₂₀ treatments. In addition, a highly significant ($P<0.001$) difference in MRGR was observed between aphids fed on T₄₀ and control treatments, as well as

between T_{80} and T_{160} treatments.

Figure 2.10 shows the mean relative growth rates of the nymphs measured at 72, 120 and 156 hours of feeding on the glycoalkaloid containing artificial diets. There was a highly significant ($P<0.001$) effect of glycoalkaloids on the MRGRs measured for all of the three periods examined, mainly due to the suppressed growth rates of nymphs observed on T_{80} and T_{160} treatments. In particular between 12-72 hours of feeding, a significant ($P<0.05$) difference was observed in the MRGRs between T_0 and T_{10} to T_{40} treatments. The difference between T_0 , T_{10} , T_{20} and T_{40} to T_{80} and T_{160} treatments was highly significant ($P<0.001$).

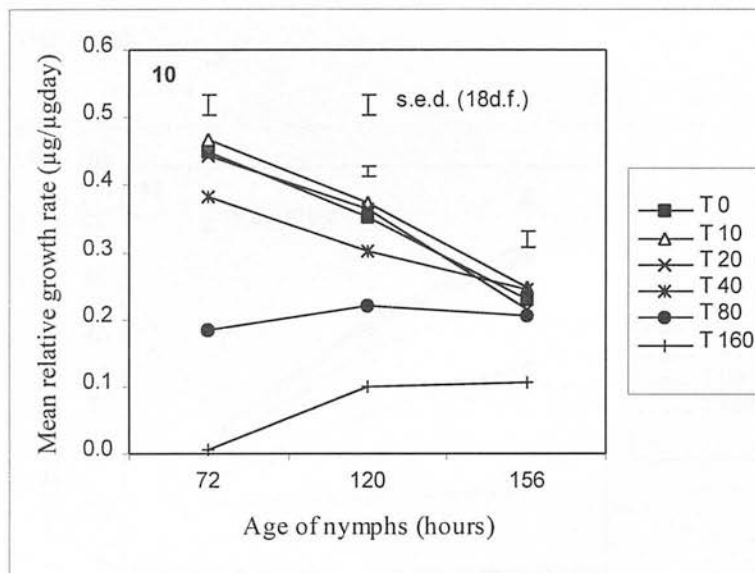


Figure 2.10. Mean relative growth rates of *Myzus persicae* nymphs reared on artificial diets containing different glycoalkaloid levels (T_0 - T_{160} mg/100ml diet) at three different time intervals (72, 120 and 156 hours of feeding).

In addition at the same period, a highly significant ($P<0.001$) difference between T_{80} and T_{160} treatments was observed. In general the MRGRs of T_0 , T_{10} , T_{20} and T_{40} treatments were decreasing as nymphs were approaching maturity (Figure 2.10). By contrast on T_{80} and T_{160} treatments, growth rates increased in the period between 72 to 120 hours and remain constant in the period between 120 to 156 hours of feeding. At the last time period (120-156 hours) of developing in pre-reproductive adults, no significant ($P>0.05$) differences were observed in the MRGRs between T_0 - T_{40} to T_{80}

treatments while the MRGR of T_{160} treatment remained significantly low ($P<0.001$).

There was a highly significant ($P<0.001$) effect of glycoalkaloid concentration of the diet, on the mean number of nymphs ecdysed per day (Table 2.3). No significant ($P>0.05$) differences were observed in the number of nymphs ecdysed per day between T_0 , T_{10} , and T_{20} treatments while in T_{40} treatment significantly ($P<0.05$) less ecdyses/day took place compared to T_0 , T_{10} , and T_{20} treatments. Highly significant ($P<0.001$) differences were observed between T_0 , T_{10} , T_{20} and T_{40} treatments to T_{80} and T_{160} treatments and between T_{80} and T_{160} treatments. Figure 2.11 shows the ecdysing progress of the nymphs measured at 72, 120 and 156 hours of feeding on the glycoalkaloid containing artificial diets, expressed as numbers of exuviae collected per treatment.

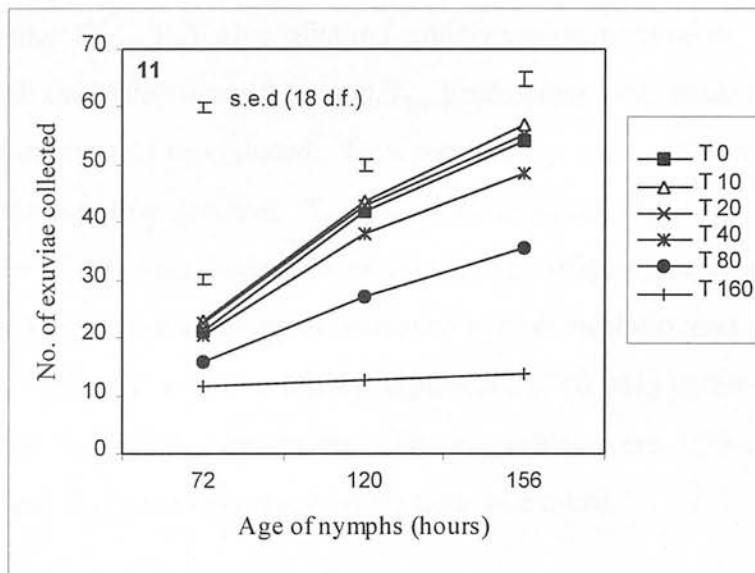


Figure 2.11. Ecdyses of *Myzus persicae* nymphs reared on artificial diets containing different glycoalkaloid levels (T_0 - T_{160} mg/100ml diet) in 72, 120 and 156 hours of feeding.

After 72 hours of feeding no significant ($P>0.05$) differences were observed in the numbers of exuviae collected between T_0 , T_{10} , T_{20} and T_{40} treatments. A significant ($P<0.05$) difference was observed between T_{40} and T_{80} treatments as well as between T_{80} and T_{160} treatments. The difference between T_0 and T_{80} treatments was significant ($P<0.01$) while between T_{10} and T_{20} to T_{80} treatments highly significant ($P<0.001$). A highly significant ($P<0.001$) difference was observed as well between T_0 , T_{10} , T_{20} , T_{40}

treatments to T_{160} treatment.

In the next time period (120 hours) a highly significant ($P < 0.001$) difference was observed between T_0 , T_{10} , T_{20} and T_{40} treatments to T_{80} and T_{160} treatments. In addition a highly significant ($P < 0.001$) difference was observed between T_{80} and T_{160} treatments. No significant differences ($P > 0.05$) were observed between T_0 , T_{10} , and T_{20} treatments, while significantly ($P < 0.05$) less exuviae were collected in T_{40} treatment compared to these collected on T_{10} and T_{20} treatments. There was no significant ($P > 0.05$) difference between T_0 and T_{40} treatments. Similar results were observed after 156 hours of feeding.

Additional data from the nymph experiment (Table 2.3 and Figure 2.12) show that the intrinsic rate of natural increase, r_m , of *M. persicae* was similar ($P > 0.05$) among control and low (T_{10} , T_{20}) glycoalkaloid concentration treatments. The nymphs generally (with the exceptions of T_{80} and T_{160} treatments) took about 6.5 days before they become adults and reproduced. However the r_m values differed significantly ($P < 0.001$) between low (control, T_{10} , T_{20}) and medium (T_{40} , T_{80}) to high (T_{160}) concentrations of glycoalkaloids. In particular, no offspring were produced from aphids fed on T_{160} treatment owing to increased nymph mortality and delayed nymph development, while there was a highly significant ($P < 0.001$) difference in the r_m values between T_{40} and T_{80} treatments. The r_m values were 1.09 and 1.51 times lower on T_{40} and T_{80} treatments respectively than on control.

Glycoalkaloids in the diet affected significantly ($P < 0.001$) the weight of the new born nymphs (Fig. 2.12). In particular, nymphs born on control, T_{10} , and T_{20} treatments were significantly ($P < 0.05$) heavier than those nymphs on T_{40} treatment. The effects of glycoalkaloids were highly significant ($P < 0.001$) between T_{80} and control to low glycoalkaloid treatments (T_{10} , T_{20}). In addition significant ($P < 0.01$) differences were found in nymph weight between T_{40} and T_{80} treatments.

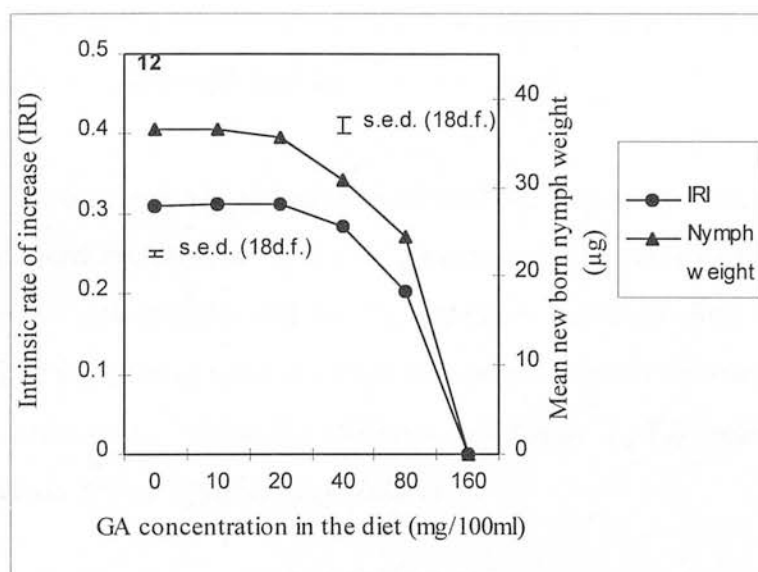


Figure 2.12. Reproductive performance of adults reared on artificial diets containing different glycoalkaloid levels (T_0 - T_{160} mg/100ml diet) and weight of new born *Myzus persicae* nymphs.

Table 2.4 presents data from cumulative mortality of the nymphs in three different time intervals. During the first time period (after 2.5 days of feeding), there was a significant ($P < 0.01$) effect of the glycoalkaloid presence in the diet due to the increased number of dead nymphs found on T_{160} treatment.

Table 2.4. Mortality, of *Myzus persicae* nymphs reared on artificial diets containing different concentrations of glycoalkaloids (GAs).

GA concentration mg/100ml	Nymph mortality after 2.5 days (%)	Nymph mortality after 4.5 days (%)	Nymph mortality after 6.5 days (%)
T_0	5.00	5.00	5.00
T_{10}	1.67	3.32	3.32
T_{20}	0.00	0.00	0.00
T_{40}	6.67	6.67	6.67
T_{80}	3.35	5.02	8.27
T_{160}	20.00	43.30	58.30
SED	4.51	4.08	4.04
DF	18	18	18
P	**	***	***

SED: standard error of difference between means, DF: degrees of freedom

** $P < 0.01$, *** $P < 0.001$

No significant ($P > 0.05$) effects were observed in nymph mortality between T_0 - T_{80} treatments with the highest –although non significant ($P > 0.05$)- mortality observed in T_{40} treatment. The difference between T_0 , T_{40} and T_{80} treatments to T_{160} treatment

was significant ($P < 0.01$) while the difference between T_{10} and T_{20} treatments to T_{160} treatment highly significant ($P < 0.001$).

In the next two time periods examined (4.5 and 6.5 days), the percent cumulative mortality remained the same in T_0 , T_{20} , T_{40} treatments and increased in T_{10} , T_{80} and T_{160} treatments. All nymphs fed on T_{20} treatment survived until maturity. No significant ($P > 0.05$) effects were observed on nymph mortality between T_0 , T_{10} , T_{20} , T_{40} and T_{80} treatments, while the difference between T_0 - T_{80} treatments to T_{160} treatment became highly significant ($P < 0.001$).

2.4 Discussion

The length of time required for an aphid to develop from birth to adult, the final adult weight, the reproductive rate and survival are dependent on food quality (Dixon, 1987). In our experiments, ingestion of an artificial diet containing 40 mg/100ml or greater of potato glycoalkaloids suppressed the reproductive performance (cumulative fecundity and intrinsic rate of increase), decreased the final weight of the pre-reproductive adults and delayed the development of nymphs to maturity, decreasing the weight gain of the nymphs. Nymphs reared on T_0 , T_{10} , T_{20} and T_{40} treatments completed their development and approached final body size and maturity earlier compared to those reared on T_{80} and T_{160} treatments. This possibly explains the increased growth rates observed between 72 and 120 hours in T_{80} and T_{160} treatments (Fig. 12a).

Elevated levels of glycoalkaloids in the diet reduced the number of nymphs born daily, as well as the number of nymphs produced during the entire aphid reproductive period which lasted 24 days (when the last born nymph was recorded). The reason for this elongated duration of measurements is that the reproductive potential of an aphid is formed in the very early stages of nymphal development and a possible effect of glycoalkaloids on fecundity might not be detected by short term experimentation. Dixon (1987), suggested that embryos of a parthenogenetically

reproducing aphid can have embryos developing within them. The glycoalkaloid presence in the diet reduced 1.09 and 1.51 times the intrinsic rate of natural increase on T_{40} and T_{80} treatments respectively compared to control, while no offspring was produced in the highest glycoalkaloid treatment. In this treatment adult longevity was affected ten days after feeding on the diet, while the effects on nymphal longevity were observed earlier. This difference might be explained by the fact that in the adult experiment the aphids used had developed their nymphal stages on Chinese cabbage leaves, and it is therefore possible that the aphids had a higher tolerance compared to the nymphs used in the nymph experiment.

Marek (1961), suggested that *Myzus ascalonicus* (Donc) appeared to prefer to feed on buffer solutions of a pH-gradient ranging between 6.2 and 8.4 rather than on solutions of a higher or lower pH. In addition other aphid species perform well on alkaline diets. The polyphagous *Aphis gossypii* Glover can accept artificial diets with pH values ranging from 6.5 to 8.0 (Auclair, 1969) and *Schizaphis graminum* (Rondani) fed on diets of pH values up to 8.5 (Dreyer *et al.*, 1981). In our experiments, *M. persicae* adapted well to the acidic (6.0) pH of the artificial diet, in contrast to the results of Schoonhoven & Derksen-Koppers, (1976). They attributed a deterrent effect of their artificial diets on the feeding of *M. persicae* to the acidity caused by the incorporation of tannic acid in the diets. The difference between our results and those of Schoonhoven & Derksen-Koppers, (1976) might be explained more by the repellent effect of tannic acid on aphid feeding than the acidity of the diets *per se*. An additional reason may be the possible differences between the biotypes of *M. persicae* used in our experiments and those that took place in the Netherlands. Furthermore, the fact that the aphids used in our experiments completed their final ecdysis in cages containing pure artificial diet (for adaptation purposes), might also have contributed to the differing results.

The same authors reported that α -solanine did not have a stimulatory effect when the artificial diet contained this substance in saturated concentration. They concluded that *M. persicae* nymphs showed normal survival after four days of feeding on the

same diet. In the experiments described adult apterae reared on T₁₀ and T₂₀ diets produced higher daily offspring compared to the control, indicating that there might be a short term stimulatory physiological role of glycoalkaloids when they are presented to the aphids in low and not high (saturated) concentrations. This possibility is supported by the fact that diet uptake was highest in the lowest glycoalkaloid treatment (T₁₀). In addition, the elevated mortality of nymphs and the delay/cancellation of maturity that were observed in our experiments suggest that the glycoalkaloids negatively affect nymphal development when they are presented to the nymphs in elevated concentrations.

Güntner *et al.* (1997), found no strong lethal effects of α -solanine and α -chaconine when offered to the potato aphid *Macrosiphum euphorbiae* (Thomas) in concentrations up to 500 ppm (equivalent to 50mg/100g FW), while α -chaconine stimulated its feeding at low concentrations. Therefore they concluded that the tolerance to glycoalkaloids that *M. euphorbiae* was observed to exhibit, is an indication that this aphid is well adapted to these substances. Our results, however, showed that *M. persicae* life parameters are negatively affected by diet concentrations similar to the medium and high glycoalkaloid concentrations (80-160 mg/100 FW equivalent to 800 and 1600 ppm respectively) which can typically be detected in potato leaves (Dao & Friedman, 1996). A possible reason for this difference observed between the two species might be explained either by a higher tolerance of *M. euphorbiae* to potato glycoalkaloids, or -most likely- by the fact that the glycoalkaloid concentrations used in the *M. euphorbiae* experiments (50-500 ppm) represent only the lower range of concentrations found in potato foliage. Comparative studies using artificial diets containing equal concentrations of glycoalkaloids may be essential to indicate in more detail the differences in life parameters between these two aphid species.

Tingey & Sinden (1982), concluded that lack of significant correlation between total glycoalkaloid concentrations in accessions of *S. berthaultii*, (Hawkes) *S. berthaultii* X *S. Tarijense*, (Hawkes) and peach potato aphid resistance levels did not

conclusively rule out the involvement of glycoalkaloids as a resistance factor to these pests. Griffiths *et al.* (1978), suggested that the plant tissues lying beneath the leaf surface but external to the phloem deserve more attention in studies on host plant selection. The route of aphid stylets to the phloem may be intercellular or intracellular or intramural (Kimmins, 1986). However today is believed that the intracellular route should be regarded more frequent (Tjallingii, 1985; Spiller *et al.*, 1985; Al-Mousawi *et al.*, 1983). *M. persicae* and in particular the first instar larvae were regarded as phloem as well as spongy mesophyll feeders (Lowe, 1967). Tjallingii (1985), using electrical penetration graphs, suggested that the short potential drops frequently produced during the stylet pathway to the phloem sieve elements -which are considered as the feeding sites of this aphid- correspond to intracellular stylet punctures through the epidermal or the mesophyllic cell membrane. There is also evidence that ingestion occurs before the aphid stylet has reached the final feeding site (McLean & Kinsey, 1967; Bradley, 1952).

It is therefore believed (Niemeyer, 1990; Harrewijn, 1990) that an aphid can locate plant resistance factors at the successive levels of the tissues encountered from the epidermis to the phloem, or can monitor the chemistry of the substrate and detect chemical gradients (van Emden, 1972); such an intake of sap samples for sensory purposes, however, has not been documented so far (Tjallingii, 1995). It is possible that during probing and/or feeding, *M. persicae* “samples” glycoalkaloids among other chemicals in the ingested sap, using the epipharyngeal organ which is in direct contact with fluid in the food canal and has the typical structure of a contact chemoreceptor (Wensler & Filshie, 1969).

Although the actual time needed for the stylets to penetrate the tissues from the epidermis to a sieve element is considered to be only a small proportion of the overall time required for sap feeding (Tjallingii, 1995), these insects require a minimum of 15 min to reach the phloem (Auclair, 1963). Therefore it seems that by feeding on phloem, aphids do not escape ingesting or coming into contact with a plant's defensive chemicals (Dixon, 1985), and in particular with glycoalkaloids in

concentrations present in the mesophyll. However the actual concentration of glycoalkaloids in the mesophyllic cells is not known. It follows therefore that the glycoalkaloid amounts imbibed by the aphid before the aphid stylet reaches the final feeding site, are not known. Furthermore, the fact that very low amounts of glycoalkaloids stimulate food uptake, suggests that glycoalkaloids may even have an indicating and/or stimulating effect on stylet penetration towards the phloem serving the aphid on host plant recognition.

It has been suggested (Roddick, 1982) that although glycoalkaloid transport between root and shoot does not take place in potato, the transport of these substances is possible at the subcellular level, especially between the site of synthesis/glycosylation (probably particulate) and the site of accumulation (probably vacuolar). This is an indication that glycoalkaloids are not present in the phloem, but further phloem sap analyses are required to document this hypothesis. It is appreciated that the studies reported in this chapter are essentially toxicological. Implications for field populations of *M. persicae*, should also be addressed. Whether glycoalkaloids act as resistance factors in the potato plant against the peach potato aphid or whether this insect has the ability to avoid inducing their production *in situ*, remain unresolved questions.

Chapter 3

Effects of nitrogen fertilisation and pesticide application, on the foliar glycoalkaloid production of potato

3.1 Introduction

The alkaloid content of the *Solanum* species varies, being influenced not only by genetic characteristics and environmental factors (climate, location, daylength), but also by different cultivation management factors including fertilisation and application of agricultural chemicals. Substantial research has been performed around several of these cultivation practices aimed at outlining their relationship with glycoalkaloid production. The conclusions reached by different researchers are often in direct conflict.

Since alkaloids are nitrogen-containing compounds, the availability of nitrogen is expected to play an important role in the biosynthesis and accumulation of alkaloids in plants. The effects of nitrogen fertilisation on tuber glycoalkaloid synthesis have been repeatedly investigated for different potato cultivars. An early study by Hutchinson & Hilton (1955), failed to reveal any significant correlation between fertiliser and fertiliser plus manure treatments applied to the Netted Gem (Russet Burbank) potato variety and solanine production in the tubers. Additional studies however, proved that excessive nitrogen fertilisation applied during potato cultivation is associated with elevated amounts of solanine produced in the tubers. In particular, fresh and stored tubers of the -withdrawn in the USA- potato cv. Lenape grown in high nitrogen plots were significantly ($P<0.05$) higher in solanine compared to those derived from normal nitrogen plots (Cronk *et al.*, 1974). These results were confirmed by Mondy & Munshi (1990), who reported significant ($P<0.05$) increases in the total glycoalkaloid tuber level, by increasing nitrogen fertilisation for all of the six potato cvs tested (Katahdin, Chipbelle, Rosa, Russet Burbank, Lemhi Russet and Shepody). Recently Love *et al.* (1994), concluded that high rates of nitrogen fertilisation applied on potato cvs Gemchip, Norchip and Russet Burbank, increased tuber glycoalkaloid concentration. However there is very limited and conflicting literature concerning the effects of nitrogen availability on potato foliar glycoalkaloids. Nowacki *et al.* (1975), suggested that the glycoalkaloid content in the dry matter of potato leaves and tops (cvs Uran, Lenino, Pionier, Baltyk and Flora)

declined after applying elevated amounts of nitrogen. By contrast Ahmed & Müller (1979), concluded that a higher level of nitrogen applied on potato cv. Grata induced an increase in α -solanine and α -chaconine concentration in the leaves and a simultaneous depression of these substances in the tubers.

Wilson & Frank (1975), studied the effects of three systemic insecticide-nematicides (disulfoton, aldicarb, carbofuran) and three systemic fungicides (thiabendazole, benomyl, thiophanate methyl) -applied before tuberisation in the field- to potato cultivars Cobbler, Katahdin and B5141-6 (withdrawn Lenape). The total glycoalkaloid tuber content of the carbofuran treatment was found to be significantly lower ($P<0.05$) compared to controls. No significant ($P>0.05$) effects of the rest of the systemic pesticides or fungicides on the total glycoalkaloid level of the tubers were observed. In addition, a greenhouse study showed that when carbofuran was applied during tuberisation to the varieties Norland, Kennebec, and Abnaki, it produced a significant increase ($P<0.01$) in the total glycoalkaloid tuber content. Wilson & Frank (1975), suggested that since the tuber total glycoalkaloid content was lower in carbofuran treated plants compared to controls, there might be a relationship between the activity of carbofuran and the biochemical pathway for glycoalkaloid biosynthesis. If glycoalkaloids play a role in potato resistance to insects, the mode of action of a systemic pesticide could be enhancement of glycoalkaloid levels, thereby providing this protection. By contrast Weingartner *et al.* (1976), found no significant differences in the total glycoalkaloids measured in tubers derived from potato plants (cvs Sebago, Pungo and Wauseon) previously treated with the insecticide-nematicides aldicarb, carbofuran, phenamiphos, oxamyl, ethoprop and fensulfothion, when those were applied in different concentrations. However no work has been done concerning the possible effects that pesticide application may have on foliar glycoalkaloids. Although these are just some of the conflicting reports found in the literature, the reasons for these discrepancies have not been explained. This study was conducted to determine how glycoalkaloids present in the potato foliage are affected by modifications of nitrogen fertilisation and pesticide application as well as the interactions between them.

3.2 Materials and Methods

Two experiments were conducted in the plant growth unit of SAC. In the first experiment (exp. 1) the potato variety King Edward was grown in three plant growth cabinets, between March – May 95. In the second experiment (exp. 2) the potato varieties King Edward and Maris Piper were grown in a plant growth room, between March – May 96. These varieties were preferred because they were considered (Percival, personal communication) as having the ability of producing high and low levels of glycoalkaloids respectively. The same growing media (with slight modifications) and nutrient solutions were used for both experiments.

3.2.1 Growing media

The potato plants were grown in a 'semi-hydroponic' culture system, using perlite as a plant growing substrate and nutrient solutions. Pots (diameter: 15cm; depth: 14cm) were stood on round (diameter: 15 cm, depth 1.5cm - exp. 1), or rectangular (22cm x 16.5cm x 4.5cm - exp. 2) trays on a glasshouse bench and filled with 1:1 mix (by volume) of horticultural grade and special seed grade perlite. Filter paper was placed in the bottom of each pot to prevent perlite leakage from the pot to the tray.

The semi-hydroponic culture system of potato plants using perlite, was preferred to that of soil or peat. Perlite as an inert material has a number of advantages over organic substrates such as peat or soil (Hall & Smith, 1991). The most significant is the lack of organic matter and the very low microbial population due to sterile conditions at the point of production. After planting, the mineral gradually becomes contaminated with root debris, mucilage and other soluble materials exuded by the roots (Lambers, 1987). These organic materials form a readily-respired substrate for micro-organisms which build up in the rhizosphere (Szmidt *et al.*, 1989). However, plant roots in perlite do not suffer the strong competition from micro-organisms for oxygen that they do in peat and other organic media (Jackson *et al.*, 1984). This situation offers the opportunity to simplify water management of perlite-grown plants by standing pots permanently in a shallow depth of water (Hall & Smith, 1991). One

disadvantage of perlite compared with peat or soil is its insignificant buffering capacity (Hall & Smith, 1994). For this reason ground magnesian limestone (1.5g/l perlite) was added separately in each pot and mixed to the perlite. The trace element availability was supported by adding fritted trace elements (WM 253A, 0.5g/l perlite). The provision of a high content of the liquid feed nitrogen in ammonium form, induced the roots to release H^+ leading to a localised low pH in the rhizosphere (Mengel & Kirkby, 1987), thus ensuring trace element availability.

3.2.2 Plant material

Potato tubers (*Solanum tuberosum* L. cv. King Edward in exp. 1 and cvs King Edward and Maris Piper in exp. 2) were layered at 3cm depth in a seed tray with perlite, placed in a plant growth cabinet at 20°C, 50% R.H., long day (16:8) conditions and watered to break dormancy. At 15 days after planting, small tuber pieces of approximately similar size with developing potato sprouts, were excised from the mother tubers using a knife. This technique was developed to reduce the possible nutritious influence of mother tubers.

Potato tuber pieces developing one sprout per tuber piece (surplus sprouts were excised), were placed at 3cm depth in the pot-gravel tray system described above, covered with perlite mix and watered with previously prepared nutrient solutions. Growing one or two sprouts / stems per pot rather than three or more –which is considered as the physiological way of potato growing- was preferred since essential manipulations for the experiment (watering or random relocation of the pots) would possibly have induced damage to the plants due to the restricted space of the plant growth cabinets and plant growth room. The pot surface was covered with black plastic around the growing potato plant to deter or reduce algal growth. Pots with developing potato sprouts were placed in three plant growth cabinets (exp. 1) and in a plant growth room (exp. 2) at 20°C, 50% R.H., and long day (16:8) conditions.

3.2.3 Nutrient solution and pesticide application

Four nutrient solutions differing in N content were combined with control (no

pesticide) and two different aldicarb levels and applied as treatments in both experiments. In exp. 1 six replicates were used per treatment while in exp. 2 four replicates were used per treatment. The nitrogen (N) treatments were N₁, N₂, N₃, N₄, representing nitrogen concentrations in the nutrient solutions of 50, 100, 200 and 300 mg total N/l respectively. Table 3.1 shows the composition of the four different nutrient solutions applied. Initially three stock solutions (A, B, and C) of final volume 1l each, were prepared separately for each nitrogen treatment.

Table 3.1. Recipes for stock solutions (g/l) diluted approximately 100 times to prepare the feeds applied to potato plants

	stock	N ₁	N ₂	N ₃	N ₄
Ammonium nitrate	A				23.10
Ammonium sulphate	A	4.90	9.75	9.95	
Calcium nitrate (12%N)	B	33.35	66.65	88.25	88.25
Mono-ammonium phosphate	A			16.35	16.35
Mono-potassium phosphate	A	17.55	17.55		
Potassium nitrate	A			38.65	66.95
Magnesium sulphate (Epsom)	A	45.00	45.00	45.00	45.00
Potassium sulphate	A	55.50	55.50	34.00	10.15
Calcium sulphate	C	40.60	15.95		

N₁: 50 mg total nitrogen/l solution, N₂: 100 mg total nitrogen/l solution, N₃: 200 mg total nitrogen/l solution, N₄: 300 mg total nitrogen/l solution

¹Calcium sulphate was added separately to the final solution as a third stock solution

The final solutions applied to potato plants were formed by diluting 100ml of each stock solution (A, B, and C) in 10 l of distilled water. The composition of the diluted feeds when applied on the treatments after dilution is shown in table 3.2.

Table 3.2. Composition of diluted feeds (mg/l) applied to potato plants after approximately 100x dilution

	N ₁	N ₂	N ₃	N ₄
Ammonium nitrogen (NH ₄ -N)	10	20	40	60
Nitrate nitrogen (NO ₃ -N)	40	80	160	240
Nitrogen (N)	50	100	200	300
Phosphorus (P)	40	40	40	40
Potassium (K)	300	300	300	300
Magnesium (Mg)	45	45	45	45
Calcium (Ca)	150	150	150	150
Sulphate sulphur (SO ₄ -S)	255	221	153	86

N₁: 50 mg total nitrogen/l solution, N₂: 100 mg total nitrogen/l solution, N₃: 200 mg total nitrogen/l solution, N₄: 300 mg total nitrogen/l solution

In exp. 1 the total amounts of nitrogen added were not measured. In exp. 2 the total amounts of nitrogen added were 150, 200, 400 and 600 mg /tray. Phosphorus and potassium applications remained constant for all four nitrogen treatments. Equal volumes of distilled water were added to all replicates to reduce condensation of nutrient solutions due to evaporation. In all the feeds the NO_3^- to NH_4^+ ratio was the same at 4:1. This was ensuring a tendency for slight acidification of the growing media which was counteracted by the lime in the perlite mix. Aldicarb was added in amounts of 0 (control P_0), 0.13g/pot (P_1), equivalent to the recommended amount/ha and 0.39g/pot (P_2), equivalent to three times the recommended amount/ha. Pots were randomly relocated in both experiments every second day. The growing potato plants were supported using wooden sticks. During the experiment dead and accidentally excised or damaged leaves and petioles were collected and placed in the deep freeze until the final harvest.

From a statistical perspective exp. 1 was a replicated two factor experiment with nitrogen applied at four levels and aldicarb applied at three levels. The treatment combinations were repeated six times. In total 72 pots were placed in the plant growth cabinets (exp. 1). The replicates were uniformly dispersed (two replicates / treatment / per plant growth cabinet). Accidentally four plots (plants) were damaged during the experiment and produced very small amounts of tissue which was insufficient to allow glycoalkaloid analysis. The data were analysed using the General Linear Model procedures of MINITAB® (vs.11.1). It was necessary to use this procedure rather than the “Analysis of Variance” in order to deal with the missing values. The output of this procedure was an analysis of variance, plus tables of means with standard errors. The differences between means measured by t-test. Exp. 2 was a replicated three factor experiment with nitrogen applied at four levels and aldicarb applied at three levels, in two potato cultivars King Edward and Maris Piper. The treatment combinations were repeated four times. In total 96 pots were placed in the plant growth room. The data were analysed using the Analysis of Variance procedure of MINITAB® (vs.11.1). The output of analysis of variance, was tables of means with standard errors. The differences between means were measured by t-test.

3.2.4 Sample preparation

Potato leaves, petioles and growing tops were excised from the stem of each plant, weighed (fresh weight FW = green matter) and placed in a white muslin sachet (one plant/sachet). All potato foliage plus developing tops and secondary suckers were harvested. Dead leaves and petioles were collected during the experiment and then were added to the finally collected batch. Each sachet was then labeled and placed in the deep freeze (exp. 1) or immersed in a container with liquid nitrogen (exp. 2). Liquid nitrogen was used to reduce further enzymatic activity which could alter the soluble nitrogen (nitrate, amino acid) as well as glycoalkaloid composition of the sampled tissues. The tissues remained in the freezer (-40°C) overnight prior to freeze-drying.

Lyophilisation took place in an Edwards freeze dryer (model: Super Modulyo) for approximately 72h/sample. Freeze-drying was preferred because of its advantages compared to oven-drying. According to Dao & Friedman (1996), it stops enzyme-catalysed, wound-induced and moisture-dependent compositional changes which may affect glycoalkaloid content; it permits storage and transportation of samples for analysis at different time periods; it allows analysis of other potato constituents. Sachets were packed under vacuum using a Vac-Elut vacuum sealer (Salton) and stored in the freeze until grinding. Each sachet was opened and the whole of the freeze-dried tissue was placed in a plastic tray, weighed and then ground using a water cooled mill (Janke & Kunkel). Each ground sample was stored in the freezer in a small plastic container until further analysis.

3.2.5 Sample extraction and purification

The extraction and analytical techniques used were based on those of Percival (personal communication) and (Hellenas, 1986). Each freeze-dried sample (0.05g) was placed in a centrifuge tube and mixed for 30 sec with 10 ml of extraction solution, (1 litre water, 20 ml acetic acid, 5g sodium bisulphite), using a vortex mixer. Tubes were placed on a tube rotator (Stewart) for 30 min. An aliquot was clarified by centrifugation at 4000 rpm for 30 min and 5 ml of clarified extract was

placed in a Bond-elut C18 cartridge previously activated by elution with 5ml acetonitrile (ACN), followed by 5ml phosphate buffer (0.05M KH_2PO_4). The cartridge was then washed with 2.5 ml ACN/phosphate buffer, 15:85 (vol/vol), and glycoalkaloids eluted with 2.5 ml ACN/phosphate buffer, 30:70 (vol/vol). A 20 μl sample was used for analytical purposes. The Bond-elut C18 cartridges were reconditioned using LiChroprep RP-18 obtained by Merck Chemicals Co.

3.2.6. Apparatus and Reagents

High performance liquid chromatography (HPLC) analysis equipment consisted of a Spectra Physics Analytical system P1000 high pressure isocratic pump, combined with a Spectra 200 programmable wavelength detector, a Gilson 232 BIO sample injector combined with a Gilson 401 dilutor fitted with a 20 μl loop and a Spectra Physics SP4270 datajet integrator. A stainless steel Phase Separations (Phase Separations Ltd., Deeside Industrial Park, Deeside CH5 2NU) column model Spherisorb, packed with Shandon ODS2-hypersil 3 μm material was used as the analytical column. The mobile phase was acetonitrile/water: 34:66 (vol/vol) and 0.5ml/l ethanolamine adjusted to pH 4.55-4.56 with orthophosphoric acid (12% solution) using a pH electrode. The chromatograph was set to deliver solvent at 1 ml/min at 0.002 absorbance units full scale (AUFS) output. Peaks were detected by UV absorbance at 202 nm. Detection of potato glycoalkaloids requires the use of UV as chromophore within the solanidine molecule does not exist. This limits the sensitivity of detection, requiring the use of relatively high sample volumes (Edwards & Cobb, 1996). Spectra of α -solanine and α -chaconine in the adopted mobile phase show maxima at 202 and 203 nm, respectively. Therefore, 202 nm was used for quantification of glycoalkaloids. All chemicals used were 'far UV grade' obtained by Fisons.

Quantification of glycoalkaloid concentration was achieved by comparing the chromatogram peak areas from potato tissues, with those obtained from standard solutions of α -solanine and α -chaconine. The HPLC analysis system was recalibrated daily using α -solanine and α -chaconine standards (125ppm – Appendices

1 & 2). This HPLC set-up allowed detection of α -solanine and α -chaconine quantities as low as 15.6 ppm. During the mobile phase and standard short trials, or during sample analyses twin split peaks were recorded occasionally in the chromatograms (Appendix 5). They were attributed mainly to impurities which entered the column causing a blockage of the system later on. These problems were overcome by turning the column and cleaning with methanol, acetonitrile, dichloromethane, acetonitrile, methanol and mobile phase each for 20 min and performing new tests using standards.

3.2.7 Calculation for conversion of area under peak to glycoalkaloid concentration mg/100g fresh weight (FW) of leaf tissue

The glycoalkaloids α -solanine and α -chaconine which were used for the standard solution preparation were obtained (purity 95%) from Sigma-Aldrich Co Ltd, Fancy Rd, Poole, Dorset, BH17 7NH England. The standard solutions were prepared by diluting 1 mg (weighed using a Sartorius microbalance, model: Ultramicro) α -solanine and 1 mg α -chaconine in 2 ml phosphate buffer (0.05M KH_2PO_4). This dilution gave a standard of 500ppm of α -solanine and 500ppm of α -chaconine. This was diluted further to 125 ppm by double dilutions using the phosphate buffer. Fresh standard solutions of pure glycoalkaloids were made every 3 days (Percival, personal communication). Comparison of chromatogram peak areas with those obtained from the 125ppm standard solutions permitted quantification of glycoalkaloids from potato leaf tissue.

The standard 125 ppm α -solanine is area of 471005 microvolts s^{-2} (mean of 12 runs). The standard 125 ppm α -chaconine is area of 582533 microvolts s^{-2} (mean of 12 runs). The α -solanine concentration in mg/100g fresh weight (FW) was calculated by the formula:

$$\frac{\text{Sample area} \times 1250 \times \text{Dry matter}}{\text{Standard area}}$$

This formula derived from the following calculations:

As standard area of 471005 microvolts s^{-2} is equivalent to 125 ppm α -solanine, it

follows that sample area of e.g. 373881 microvolts s^{-2} will be 99.2 ppm α -solanine. This means that 1000ml of the final eluate contain 99.2mg α -solanine. Since all of the extracted α -solanine is contained in 2.5ml of the final eluate solution it follows that 2.5ml of final eluate solution contain 0.25mg α -solanine. These 0.25mg α -solanine were in the 5ml of extraction solution which derived from 0.025g of dried tissue (since 0.05g were weighed and 10ml of extraction solution were added). Since the dried tissue e.g. 2.03g derived from 15.2g fresh tissue it follows that 0.025g dried tissue derived from 0.19g fresh tissue. In conclusion 0.19g fresh weight contain the same amount of α -solanine contained in 0.025g dry weight. So if 0.19g fresh weight contain 0.25mg α -solanine it follows that 100g fresh weight (FW) contain 131.6mg α -solanine. The same formula was used for the calculation of the concentration of α -chaconine. The glycoalkaloid concentration was expressed as mg of α -solanine and α -chaconine per 100mg fresh weight (FW) and as well as mg of α -solanine and α -chaconine per 100mg dry weight (DW).

3.3 Results

Experiment 1

3.3.1 Effects of nitrogen fertilisation on green matter, dry matter, total and individual glycoalkaloids

Table 3.3a shows the effects of nitrogen fertilisation on the green matter (FW), dry matter (DW/FW) and total foliar fresh (FW) and dry (DW) weight glycoalkaloid production of potato plants. As expected there was a highly significant ($P < 0.001$) effect of nitrogen fertilisation on the amounts of green matter (fresh weight of leaves and growing tops) harvested from the plants. In particular, highly significant differences ($P < 0.001$) were observed between N_1 to N_2 , N_3 and N_4 treatments, and between N_2 to N_3 and N_4 treatments. No significant differences ($P > 0.05$) in the green matter produced, were observed between N_3 and N_4 treatments. A significant ($P < 0.01$) effect due to N fertilisation was observed as well for dry matter (dry weight to fresh weight ratio).

Table 3.3a. Effects of nitrogen (N) fertilisation on green matter, dry matter and total foliar fresh and dry weight glycoalkaloid (GA) production of potato plants cv. King Edward

Nutrient Solution	Green matter FW (g)	Dry matter DW/FW	Total GAs		
			FW (mg/100g)	DW(mg/100g)	yield (mg)
N ₁	5.8	0.15	391.7	2427.1	26.1
N ₂	12.1	0.13	252.4	1918.7	31.3
N ₃	23.3	0.12	128.2	1074.6	30.6
N ₄	24.5	0.11	137.3	1212.9	35.0
SED	1.7	0.01	42.5	167	6.2
DF	54	52	51	51	51
P	***	**	***	***	NS

SED: standard error of difference between means, DF: degrees of freedom

*** P<0.001, ** P<0.01, NS Non significant

N₁: 50 mg total nitrogen/l solution, N₂: 100 mg total nitrogen/l solution, N₃: 200 mg total nitrogen/l solution, N₄: 300 mg total nitrogen/l solution

FW: fresh weight, DW: dry weight

Plants grown in N₁ treatments produced significantly more dry matter compared to those grown in N₂ (P<0.05), N₃ (P<0.01), and N₄ (P<0.001). In addition no significant (P>0.05) differences were observed in the dry matter produced between N₂ to N₃ treatments and between N₃ to N₄ treatments, while the difference between N₂ and N₄ treatments was significant (P<0.05). There was a highly significant (P<0.001) effect of the N fertilisation on the total foliar glycoalkaloid production of potato plants, expressed as mg of glycoalkaloids produced / 100g fresh (FW) and/or dry (DW) weight (Table 3.3a).

Elevated levels of nitrogen in the nutrient solution reduced the total fresh and dry weight content (mg/100g) of glycoalkaloids and increased, although non significantly (P>0.05), the total glycoalkaloid yield per treatment (Figures 3.1 & 3.2). Plants grown in N₁ treatment produced significantly (P<0.01) higher amounts of total (Table 3.3a and Figure 3.1) glycoalkaloids expressed as mg/100g FW compared to plants grown in the N₂ treatment. The differences between N₁ to N₃ and N₄ treatments were highly significant (P<0.001), while these between N₂ to N₃ and N₄ treatments were significant (P<0.01). No significant (P>0.05) differences were observed in the total content (mg/100g FW) of glycoalkaloids produced between N₃ and N₄ treatments.

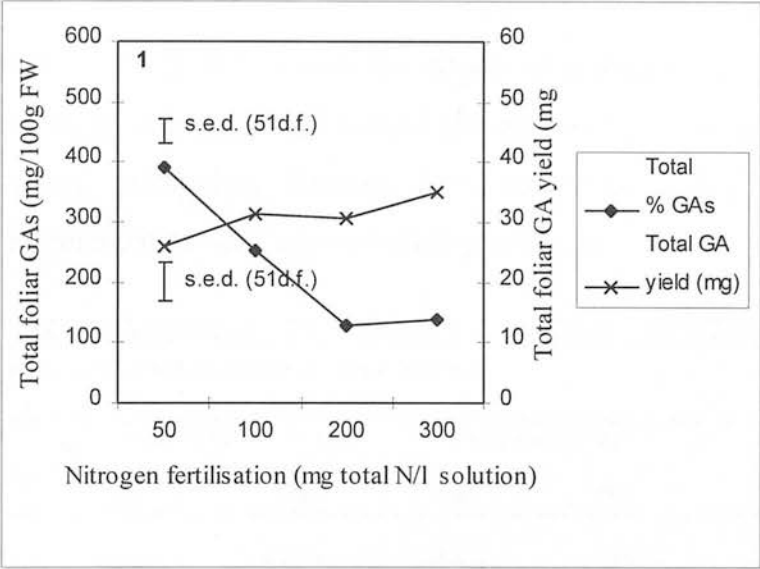


Figure 3.1. Total content (mg/100g) and total per plant (mg) foliar fresh weight (FW) glycoalkaloid (GAs) production for four nitrogen (N_1 , N_2 , N_3 and N_4) fertilisation levels applied (Exp. 1).

Similar results were observed when glycoalkaloid production was expressed in mg/100g dry weight (Figure 3.2). The difference in the total glycoalkaloids produced (mg/100g dry weight) was significant ($P<0.01$) between N_1 and N_2 treatments, while that between N_1 to N_3 and N_4 treatments was highly significant ($P<0.001$).

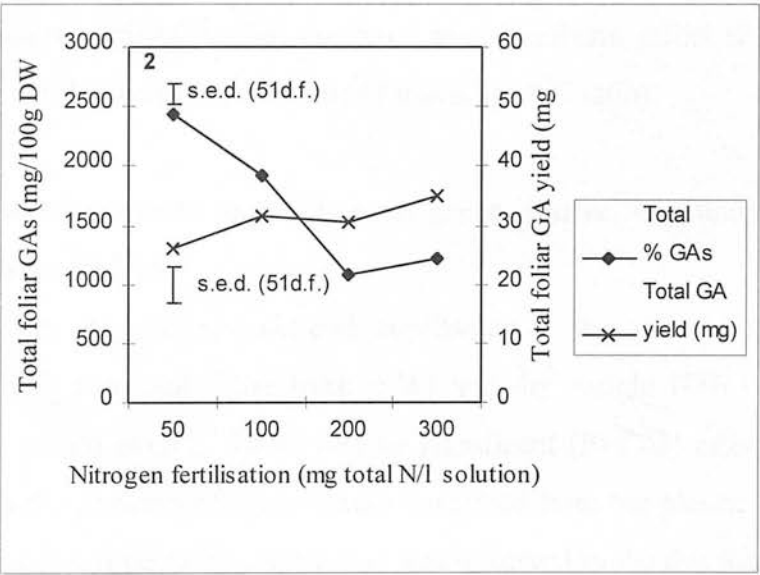


Figure 3.2. Total content (mg/100g) and total per plant (mg) foliar dry weight (DW) glycoalkaloid (GAs) production for four nitrogen (N_1 , N_2 , N_3 and N_4) fertilisation levels applied (Exp. 1).

In addition a highly significant ($P<0.001$) difference was observed between N_2 to N_3

and N₄ treatments. No significant ($P>0.05$) differences were observed between N₃ and N₄ treatments. Table 3.3b shows the effects of nitrogen fertilisation on the individual fresh (FW) and dry (DW) weight glycoalkaloid production. As for the total glycoalkaloid production, nitrogen fertilisation had a highly significant ($P<0.001$) effect on the individual glycoalkaloid production.

Table 3.3b. Effects of nitrogen (N) fertilisation on individual foliar glycoalkaloid (GA) production of potato plants cv. King Edward

Nutrient solution	α -sol (mg/100g)		α -cha (mg/100g)		S:C ratio
	FW	DW	FW	DW	
N ₁	134.9	831.9	256.8	1595.2	34:66
N ₂	86.7	658.1	165.7	1259.5	34:66
N ₃	44.1	370.0	84.0	706.8	35:65
N ₄	46.9	424.9	90.4	810.4	34:66
SED	14.9	57.5	27.6	109.6	0.02
DF	51	51	51	51	51
P	***	***	***	***	NS

SED: standard error of difference between means, DF: degrees of freedom
 *** $P<0.001$, NS Non significant
 N₁: 50 mg total nitrogen/l solution, N₂: 100 mg total nitrogen/l solution, N₃: 200 mg total nitrogen/l solution, N₄: 300 mg total nitrogen/l solution
 FW: fresh weight, DW: dry weight
 α -sol: α -solanine, α -cha: α -chaconine, S:C: ratio of α -solanine to α -chaconine

However nitrogen fertilisation did not have any significant effect ($P>0.05$) on the proportion of α -solanine to α -chaconine (Table 3.3b, S:C ratio).

3.3.2 Effects of pesticide application on green matter, dry matter, total and individual glycoalkaloids

Table 3.4a shows the effects of aldicarb application on the green matter (FW), dry matter (DW/FW) and total foliar fresh (FW) and dry weight (DW) glycoalkaloid production of potato plants. There was no significant ($P>0.05$) effect of pesticide application on the amounts of green matter harvested from the plants. A significant ($P<0.05$) effect due to pesticide application was observed in the dry matter produced. Plants grown in pesticide free pots (P₀ treatments) produced significantly ($P<0.05$) more dry matter compared to those grown with pesticide applied (P₁ and P₂ treatments).

Table 3.4a. Effects of pesticide application on green matter, dry matter, and total foliar fresh and dry weight glycoalkaloid (GA) production of potato plants cv. King Edward

Pesticide applied	Green matter FW (g)	Dry matter DW/FW	Total GAs (mg/100g)	
			FW	DW
P ₀	17.66	0.14	311.5	1978.1
P ₁	15.14	0.12	182.3	1482.2
P ₂	16.51	0.12	188.5	1514.7
SED	1.48	0.01	36.8	144.6
DF	54	52	51	51
P	NS	*	**	**

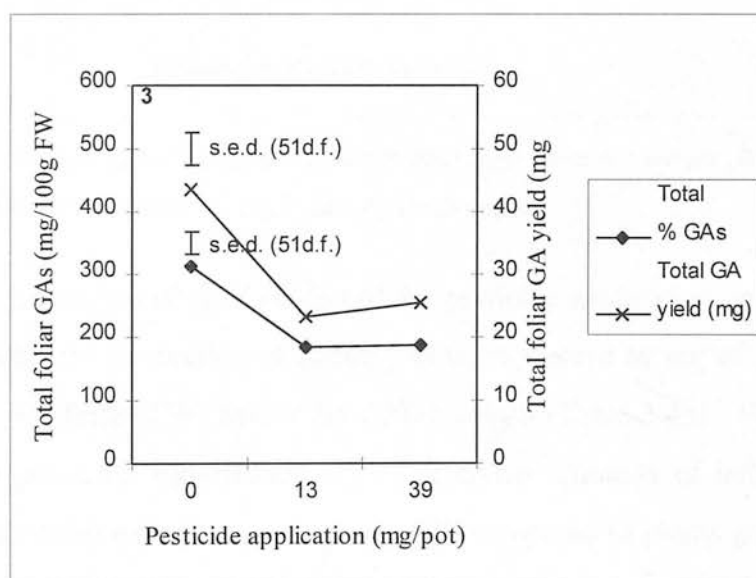
SED: standard error of difference between means, DF: degrees of freedom

** P<0.01, * P<0.05, NS Non significant

P₀: 0 mg aldicarb / pot, P₁: 0.13 mg aldicarb / pot, P₂: 0.39 mg aldicarb / pot

FW: fresh weight, DW: dry weight

There was a significant ($P<0.01$) effect of the pesticide application on the total foliar glycoalkaloid production of potato plants, expressed as mg of glycoalkaloids produced / 100g fresh (FW) and/or dry (DW) weight (Table 3.4a). Elevated levels of pesticide in the growing media (perlite) reduced significantly ($P<0.01$) not only the total fresh and dry weight content (mg/100g) of glycoalkaloids but also the total glycoalkaloid yield per treatment ($P<0.001$) (Figures 3.3 & 3.4).

**Figure 3.3.** Total content (mg/100g) and total per plant (mg) foliar fresh weight (FW) glycoalkaloid (GAs) production for three pesticide (P₀, P₁ and P₂) levels applied.

Plants grown in P_0 treatment produced significantly ($P < 0.01$) higher amounts of total (Table 3.4a and Figure 3.3) glycoalkaloids expressed as mg/100g FW compared to plants grown in P_1 and P_2 treatments. No significant ($P > 0.05$) differences were observed in the content (mg/100g) of the total glycoalkaloids produced between P_1 and P_2 treatments. Similar results were observed when glycoalkaloid production was expressed in mg/100g dry weight (Figure 3.4). The difference in the total glycoalkaloids produced (mg/100g dry weight) was significant ($P < 0.01$) between P_0 to P_1 and P_2 treatments. No significant ($P > 0.05$) differences were observed between P_1 and P_2 treatments.

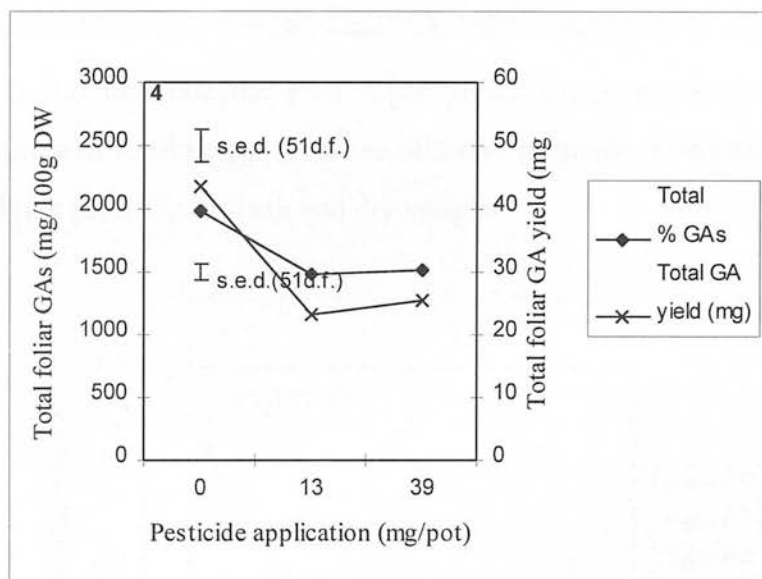


Figure 3.4. Total content (mg/100g) and total per plant (mg) foliar dry weight (DW) glycoalkaloid (GAs) production for three pesticide (P_0 , P_1 and P_2) levels applied.

There was a significant ($P < 0.01$) effect of the pesticide application on the individual foliar glycoalkaloid production of potato plants, expressed as mg of glycoalkaloids produced / 100g fresh (FW) and/or dry (DW) weight (Table 3.4b). Plants grown in P_0 treatment produced significantly ($P < 0.01$) higher amounts of individual (Table 3.4b) glycoalkaloids expressed as mg/100g FW compared to plants grown in P_1 and P_2 treatments. No significant ($P > 0.05$) differences were observed in the content (mg/100g) of individual glycoalkaloids produced between P_1 and P_2 treatments. Pesticide application did not have any significant effect ($P > 0.05$) on the proportion of α -solanine to α -chaconine (Table 3.4b S:C ratio).

Table 3.4b. Effects of pesticide application on individual foliar glycoalkaloid (GA) production of potato plants cv. King Edward

Pesticide applied	α -sol (mg/100g)		α -cha (mg/100g)		S:C ratio
	FW	DW	FW	DW	
P ₀	108.2	685.4	203.2	1291.5	35:65
P ₁	62.5	507.7	119.7	974.5	35:65
P ₂	63.8	520.5	124.7	1012.9	34:66
SED	12.9	49.8	23.9	94.9	0.015
DF	51	51	51	51	51
P	**	**	**	**	NS

SED: standard error of difference between means, DF: degrees of freedom
 ** P<0.01, NS Non significant
 P₀: 0 mg aldicarb / pot, P₁: 0.13 mg aldicarb / pot, P₂: 0.39 mg aldicarb / pot
 FW: fresh weight, DW: dry weight
 α -sol: α -solanine, α -cha: α -chaconine, S:C: ratio of α -solanine to α -chaconine

Figures 3.5 & 3.6 illustrate the total foliar glycoalkaloid production as this was affected by nitrogen fertilisation for three different pesticide levels expressed in mg of glycoalkaloids per 100g of fresh and dry weight.

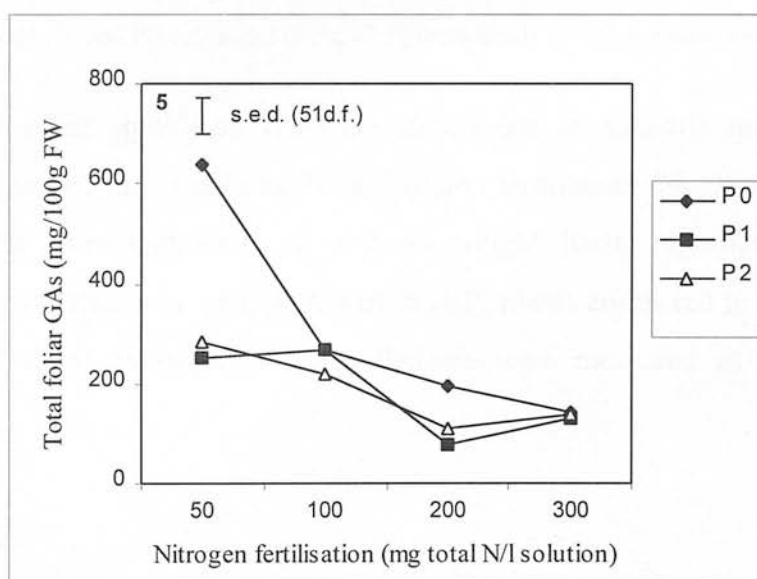


Figure 3.5. Total foliar glycoalkaloid production affected by nitrogen (N) fertilisation for three pesticide levels (P₀, P₁ and P₂) expressed in mg of glycoalkaloids per 100g of fresh weight (FW).

The significant (P<0.01) effect of pesticide application on glycoalkaloid production observed (Table 3.4a), was mainly due to the highly significant (P<0.001) elevated amounts of glycoalkaloids produced on the N₁P₀ treatment, compared to those produced on N₁P₁ and N₁P₂ treatments (Figure 3.5). Similar results observed when

glycoalkaloid production was expressed in dry weight basis where plants grown in N_1P_0 treatment, produced significantly more glycoalkaloids compared to those produced in N_1P_1 ($P<0.001$) and N_1P_2 ($P<0.01$) treatments (Figure 3.6)

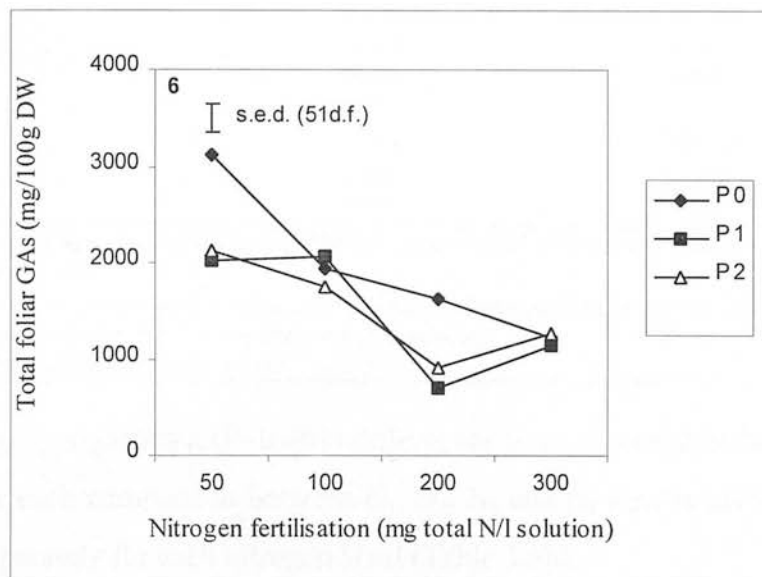


Figure 3.6. Total foliar glycoalkaloid production affected by nitrogen (N) fertilisation for three pesticide levels (P_0 , P_1 and P_2) expressed in mg of glycoalkaloids per 100g of dry weight (DW).

Although no other significant ($P>0.05$) effects due to aldicarb application were observed in any of the remaining N fertilisation treatments (N_2 , N_3 and N_4) when glycoalkaloids were expressed on a fresh weight basis, significantly elevated amounts of glycoalkaloids were produced in N_3P_0 plants compared to N_3P_1 ($P<0.01$) and N_3P_2 ($P<0.05$) plants when glycoalkaloids were measured as mg/100g DW (Figure 3.6).

Experiment 2

3.3.3 Effects of nitrogen fertilisation on green and dry matter

Tables 3.5a, 3.5b, and 3.5c show data from exp.2. There was a highly significant ($P<0.001$) overall effect of N fertilisation on green matter harvested for both varieties. In particular, plants from both varieties grown in N_1 treatments produced significantly ($P<0.001$) less green matter compared to that produced in N_2 , N_3 and N_4 treatments (KE+MP means, Table 3.5a). In addition the differences between N_2 , to N_3 and N_4 treatments were all highly significant ($P<0.001$) (Table 3.5a).

Table 3.5a. Effects of nitrogen (N) fertilisation on green and dry matter production of potato plants cvs. King Edward and Maris Piper

Nutrient solution	Green matter FW (g)	Dry matter DW/FW
N ₁	16.20	0.13
N ₂	40.90	0.12
N ₃	74.00	0.10
N ₄	99.10	0.09
SED	1.64	0.002
DF	69	69
P	***	***

SED: standard error of difference between means, DF: degrees of freedom
 *** P < 0.001
 N₁: 50 mg total nitrogen/l solution, N₂: 100 mg total nitrogen/l solution, N₃: 200 mg total nitrogen/l solution, N₄: 300 mg total nitrogen/l solution
 FW: fresh weight, DW: dry weight

The same highly significant ($P < 0.001$) differences were observed in the green matter produced for each comparison between N₁, N₂, N₃ and N₄ treatments in each variety examined separately for each nitrogen level (Table 3.5b).

Table 3.5b. Interaction effects of nitrogen (N) fertilisation x cultivar on green and dry matter production of potato plants cvs. King Edward (KE) and Maris Piper (MP)

Nutrient solution	Green matter FW (g)		Dry matter DW/FW	
	KE	MP	KE	MP
N ₁	14.1	18.3	0.13	0.13
N ₂	43.5	38.3	0.11	0.13
N ₃	65.4	82.6	0.09	0.11
N ₄	83.8	114.3	0.08	0.10
SED	2.33		0.003	
DF	69		69	
P	***		***	

SED: standard error of difference between means, DF: degrees of freedom
 *** P < 0.001
 N₁: 50 mg total nitrogen/l solution, N₂: 100 mg total nitrogen/l solution, N₃: 200 mg total nitrogen/l solution, N₄: 300 mg total nitrogen/l solution
 FW: fresh weight, DW: dry weight

In general cv. Maris Piper harvested at the same physiological stage as cv. King Edward (prior to flowering) produced significantly ($P < 0.001$) more green matter (Table 3.5c). This was mainly due to the elevated amounts of green matter produced by cv Maris Piper compared to these produced by cv. King Edward on N₃ and N₄

treatments (Table 3.5b). No significant ($P > 0.05$) differences were observed in the green matter produced in N_1 treatment between the two cultivars. An exception was observed in N_2 treatment in which cv. King Edward produced significantly ($P < 0.05$) more green matter than that produced in cv. Maris Piper (Table 3.5b).

Table 3.5c. Effects of cultivar on green and dry matter production of potato plants cvs. King Edward (KE) and Maris Piper (MP)

	Green matter FW (g)		Dry matter DW/FW	
	KE	MP	KE	MP
	51.7	63.4	0.10	0.12
SED	1.16		0.001	
DF	69		69	
P	***		***	
SED: standard error of difference between means, DF: degrees of freedom				
*** P < 0.001				
FW: fresh weight, DW: dry weight				

A highly significant ($P < 0.001$) overall effect on dry matter due to N fertilisation was observed as in the previous experiment (exp. 1). Plants from both potato cultivars grown in N_1 treatments produced significantly ($P < 0.001$) more dry matter compared to those grown in N_2 , N_3 and N_4 treatments (KE+MP means, Table 3.5a). The differences between N_2 , N_3 and N_4 treatments were highly significant ($P < 0.001$) as well.

When the effects of nitrogen fertilisation on the dry matter production were examined separately for each potato cultivar, highly significant differences ($P < 0.001$) were observed for each comparison between all of the N_1 to N_2 , N_3 and N_4 treatments (Table 3.5b). An exception was observed in cv. Maris Piper (Table 3.5b) in which the same dry matter was produced in both N_1 and N_2 treatments. However the difference between N_3 and N_4 treatments in the same cultivar was significant ($P < 0.01$). In general cv. Maris Piper produced significantly ($P < 0.001$) more dry matter than that produced by cv. King Edward (Table 3.5c). This was mainly due to the significantly ($P < 0.001$) elevated amounts of dry matter produced in cv. Maris Piper vs. cv. King Edward in N_2 , N_3 and N_4 treatments (Table 3.5b). Both varieties produced the same amount of dry matter in N_1 treatment (Table 3.5b).

3.3.4 Effects of nitrogen fertilisation on total glycoalkaloids

Tables 3.6a, 3.6b and 3.6c and figures 3.7 & 3.8 show the glycoalkaloid production by cvs King Edward and Maris Piper as this was affected by nitrogen (N) fertilisation. As in exp. 1 there was a highly significant ($P < 0.001$) overall effect of nitrogen fertilisation on the total foliar FW or DW glycoalkaloid production of potato plants (Table 3.6a).

Table 3.6a. Effects of nitrogen (N) fertilisation on fresh weight, dry weight and total foliar glycoalkaloid (GA) production of potato plants cvs. King Edward and Maris Piper

Nutrient solution	Total GAs (mg/100g FW)	Total GAs (mg/100g DW)	Total GA yield (mg)
N ₁	282.7	2109.9	45.1
N ₂	186.0	1545.1	77.1
N ₃	89.4	864.7	67.1
N ₄	65.4	694.8	64.1
SED	12.0	85.0	5.4
DF	69	69	69
P	***	***	***

SED: standard error of difference between means, DF: degrees of freedom
 *** $P < 0.001$
 N₁: 50 mg total nitrogen/l solution, N₂: 100 mg total nitrogen/l solution, N₃: 200 mg total nitrogen/l solution, N₄: 300 mg total nitrogen/l solution
 FW: fresh weight, DW: dry weight

Elevated levels of N in the nutrient solution reduced the total glycoalkaloid synthesis when this was expressed as mg of glycoalkaloids produced / 100g fresh (FW) or dry (DW) weight (Table 3.6a and Figures 3.7 & 3.8). As presented in table 3.6a and figures 3.7 and 3.8 plants from both varieties (KE+MP means) grown in N₁ treatments produced significantly ($P < 0.001$) higher amounts of total FW or DW glycoalkaloid content (mg/100g) compared to plants grown in N₂, N₃ and N₄ treatments. The differences between N₂ to N₃ and N₄ treatments were highly significant ($P < 0.001$), while no significant ($P > 0.05$) differences were observed between N₃ and N₄ treatments. In addition there was an increase in the total glycoalkaloid yield harvested per treatment (KE+MP means, Table 3.6a and Figures 3.7 and 3.8). A highly significant ($P < 0.001$) difference was observed in the total glycoalkaloid yield between N₁ to N₂, N₃ and N₄ treatments. No significant

differences ($P>0.05$) were observed between N_2 to N_3 and N_3 to N_4 treatments. However N_2 treatment produced significantly ($P<0.05$) higher total glycoalkaloid yield to that produced in N_4 treatment.

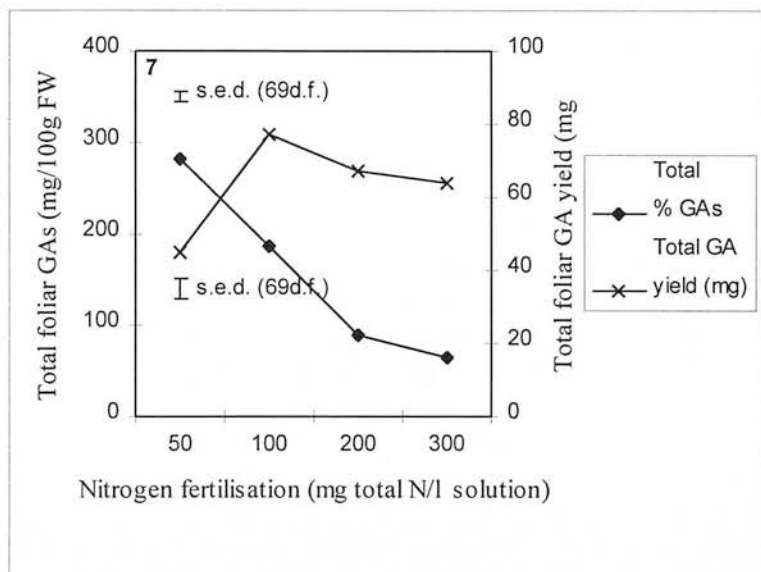


Figure 3.7. Total content (mg/100g) and total per plant (mg) foliar fresh weight (FW) glycoalkaloid (GAs) production for four nitrogen (N_1 , N_2 , N_3 and N_4) fertilisation levels applied (KE+MP means, Exp. 2).

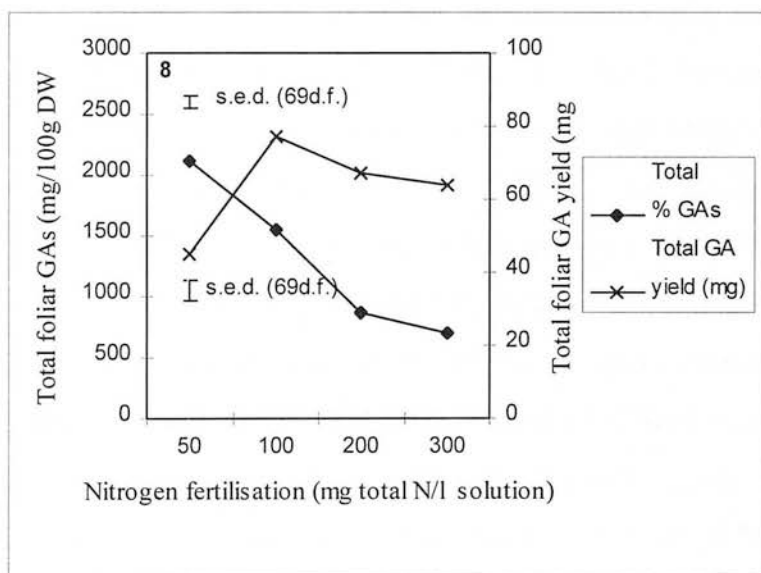


Figure 3.8. Total content (mg/100g) and total per plant (mg) foliar dry weight (DW) glycoalkaloid (GAs) production for four nitrogen (N_1 , N_2 , N_3 and N_4) fertilisation levels applied (KE+MP means, Exp. 2).

The same results were observed not only for the total glycoalkaloids produced by both varieties but also for glycoalkaloids produced separately by each variety (KE or MP) and measured against fresh and/or dry weights (Table 3.6b).

Table 3.6b. Interaction effects of nitrogen (N) fertilisation x cultivar on fresh weight, dry weight and total foliar glycoalkaloid (GA) production of potato plants cvs. King Edward (KE) and Maris Piper (MP)

Nutrient solution	Total GAs (mg/100g FW)		Total GAs (mg/100g DW)		Total GA yield (mg)	
	KE	MP	KE	MP	KE	MP
N ₁	312.1	253.3	2340.6	1879.2	44.3	45.8
N ₂	219.9	152.1	1898.1	1192.1	96.6	57.5
N ₃	97.1	81.7	995.7	733.7	66.6	67.6
N ₄	72.8	58.0	856.3	533.3	61.7	66.5
SED	17		120.1		7.6	
DF	69		69		69	
P	NS		NS		***	

SED: standard error of difference between means, DF: degrees of freedom
 *** P < 0.001, NS Non significant
 N₁: 50 mg total nitrogen/l solution, N₂: 100 mg total nitrogen/l solution, N₃: 200 mg total nitrogen/l solution, N₄: 300 mg total nitrogen/l solution
 FW: fresh weight, DW: dry weight

In general cv. King Edward produced significantly ($P < 0.001$) higher amounts of total FW or DW glycoalkaloid content (mg/100g) to that produced by cv. Maris Piper (Table 3.6c). This was due to the significantly ($P < 0.001$) elevated amounts of glycoalkaloids produced in cv. King Edward compared to that produced in cv Maris Piper in N₁ and N₂ nitrogen treatments (Table 3.6b). No significant ($P > 0.05$) differences were observed in the FW glycoalkaloid content produced between the two varieties in the N₃ and N₄ nitrogen treatments. By contrast when glycoalkaloids were measured against dry weights, the differences between the two varieties in the N₃ and N₄ nitrogen treatments were significant ($P < 0.05$) and ($P < 0.01$) respectively (Table 3.6b). A result of the reduced FW and DW content (mg/100g) of glycoalkaloids produced in cv. Maris piper, was that the total yield of glycoalkaloids produced by Maris Piper was significantly ($P < 0.05$) decreased compared to the total yield of glycoalkaloids produced by cv. King Edward (Table 3.6c).

Table 3.6c. Effects of cultivar on fresh weight, dry weight and total foliar glycoalkaloid (GA) production of potato plants cvs. King Edward (KE) and Maris Piper (MP)

	Total GAs (mg/100g FW)		Total GAs (mg/100g DW)		Total GA yield (mg)	
	KE	MP	KE	MP	KE	MP
	175.5	136.3	1522.7	1084.6	67.3	59.3
SED	8.5		60.1		3.8	
DF	69		69		69	
P	***		***		*	

SED: standard error of difference between means, DF: degrees of freedom
 *** P < 0.001, * P < 0.05
 FW: fresh weight, DW: dry weight

This was mainly due to the significantly ($P < 0.001$) elevated amounts of total glycoalkaloid yield produced by cv. King Edward in N_2 treatment compared to the glycoalkaloid amounts produced in the same nitrogen treatment N_2 by cv. Maris Piper (Table 3.6b). No significant ($P > 0.05$) differences in the total glycoalkaloid yield were observed between the two cultivars in any of N_1 , N_3 and N_4 treatments (Table 3.6b).

3.3.5 Effects of pesticide application on green and dry matter

Tables 3.7a and 3.7b show the effects of aldicarb application on the green (FW) and dry (DW/FW) matter on potato plants.

Table 3.7a. Effects of pesticide application on green and dry matter production of potato plants cvs. King Edward and Maris Piper

Pesticide applied	Green matter FW (g)	Dry matter DW/FW
P ₀	59.3	0.11
P ₁	58.9	0.11
P ₂	54.5	0.12
SED	1.42	0.002
DF	69	69
P	***	*

SED: standard error of difference between means, DF: degrees of freedom
 *** P < 0.001, * P < 0.05
 P₀: 0 mg aldicarb / pot, P₁: 0.13 mg aldicarb / pot, P₂: 0.39 mg aldicarb / pot
 FW: fresh weight, DW: dry weight

There was a highly significant ($P < 0.001$) effect of pesticide on the amounts of green

matter harvested from the plants (KE+MP means, Table 3.7a). This was mainly due to the significantly ($P<0.001$) decreased green matter produced in P_2 treatment of cv. King Edward compared to P_0 and P_1 treatments (Table 3.7b).

Table 3.7b. Interaction effects of pesticide application x cultivar on green and dry matter production of potato plants cvs. King Edward (KE) and Maris Piper (MP)

Pesticide applied	Green matter FW (g)		Dry matter DW/FW	
	KE	MP	KE	MP
P_0	53.8	64.8	0.11	0.11
P_1	54.6	63.1	0.10	0.12
P_2	46.7	62.1	0.12	0.12
SED	2.01		0.003	
DF	69		69	
P	NS		NS	

SED: standard error of difference between means, DF: degrees of freedom
 NS Non significant
 P_0 : 0 mg aldicarb / pot, P_1 : 0.13 mg aldicarb / pot, P_2 : 0.39 mg aldicarb / pot
 FW: fresh weight, DW: dry weight

No significant differences ($P>0.05$) were observed in the green matter produced between P_0 and P_1 treatments in cv. King Edward, as well as between P_0 , P_1 and P_2 treatments in cv. Maris Piper (Table 3.7b). In addition a significant ($P<0.05$) effect of pesticide was observed in the dry matter produced. This was due to the highly significant ($P<0.001$) difference observed in cv. King Edward between P_1 and P_2 treatments (Table 3.7b).

3.3.6 Effects of pesticide application on total glycoalkaloids

Tables 3.8a, 3.8b and figures 3.9 & 3.10 show the effects of pesticide application on the glycoalkaloid production of cvs King Edward and Maris Piper expressed as mg produced per 100g fresh or dry weight. There was a significant effect of pesticide on the glycoalkaloid production of potato plants when this was expressed against fresh ($P<0.01$) or dry weights ($P<0.001$) (KE+MP means, Table 3.8a, and figures 3.9 & 3.10). Plants grown in pesticide free media (P_0) produced less –although non significant ($P>0.05$)- FW glycoalkaloids compared to P_1 , and significantly ($P<0.05$) more FW glycoalkaloids compared to P_2 treatments.

Table 3.8a. Effects of pesticide application on fresh weight, dry weight and total glycoalkaloid (GA) production of potato plants cvs. King Edward and Maris Piper

Pesticide applied	Total GAs (mg/100g FW)	Total GAs (mg/100g DW)	Total GA yield (mg)
P ₀	159.3	1358.7	66.2
P ₁	173.3	1441.6	70.5
P ₂	134.9	1110.6	53.3
SED	10.4	73.6	4.7
DF	69	69	69
P	**	***	***

SED: standard error of difference between means, DF: degrees of freedom

*** P<0.001, ** P<0.01

P₀: 0 mg aldicarb / pot, P₁: 0.13 mg aldicarb / pot, P₂: 0.39 mg aldicarb / pot

FW: fresh weight, DW: dry weight

Table 3.8b. Interaction effects of pesticide application x cultivar on fresh weight, dry weight and total glycoalkaloid (GA) production of potato plants cvs. King Edward (KE) and Maris Piper (MP)

Pesticide applied	Total GAs (mg/100g FW)		Total GAs (mg/100g DW)		Total GA yield (mg)	
	KE	MP	KE	MP	KE	MP
P ₀	171.7	147.0	1531.8	1185.7	68.5	63.9
P ₁	203.9	142.7	1759.2	1123.9	82.4	58.5
P ₂	150.9	119.0	1277.0	944.2	51.0	55.5
SED	14.7		104		6.6	
DF	69		69		69	
P	NS		NS		*	

SED: standard error of difference between means, DF: degrees of freedom

* P<0.05, NS Non significant

P₀: 0 mg aldicarb / pot, P₁: 0.13 mg aldicarb / pot, P₂: 0.39 mg aldicarb / pot

FW: fresh weight, DW: dry weight

In particular cv. King Edward produced significantly ($P<0.05$) less FW or DW glycoalkaloids in P₀ treatment compared to P₁ treatment (Table 3.8b). No significant differences ($P>0.05$) were observed between the same treatments in fresh or dry weight glycoalkaloids produced by cv. Maris Piper. Both varieties produced less glycoalkaloids in P₂ treatment compared to P₀ treatment but this difference was significant ($P<0.05$) only when glycoalkaloid production was expressed against dry weights (Table 3.8b). No significant differences ($P>0.05$) were observed in the total yield of glycoalkaloids harvested between P₀ and P₁ treatments, while in P₂ treatments were produced significantly less ($P<0.001$) glycoalkaloids to those

produced in P_0 treatments (KE+MP means, Table 3.8a and figures 3.9 & 3.10). In cv. King Edward the total glycoalkaloid yield was significantly ($P<0.05$) increased in P_1 treatments and significantly ($P<0.01$) decreased in P_2 treatments compared to P_0 treatments (Table 3.8b). In addition no significant differences ($P>0.05$) were observed in the total yield of glycoalkaloids produced between any of pesticide treatments in cv. Maris Piper (Table 3.8b).

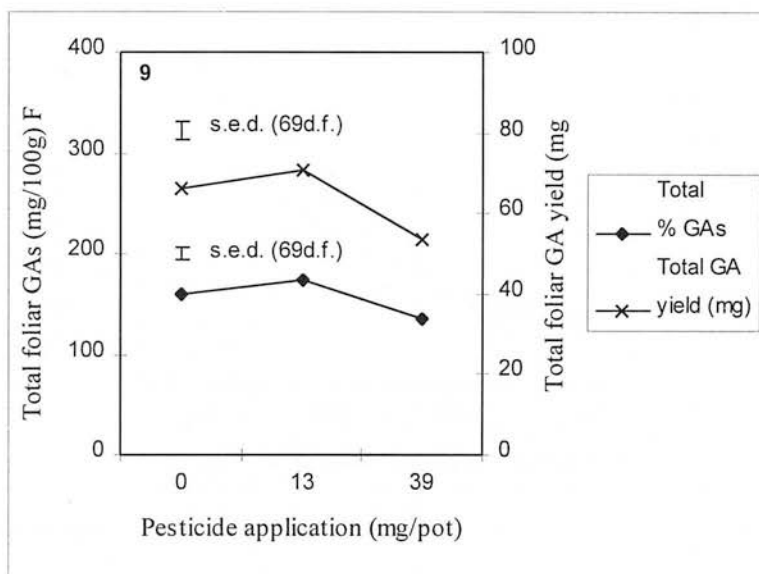


Figure 3.9. Total content (mg/100g) and total per plant (mg) foliar fresh weight (FW) glycoalkaloid (GAs) production for three pesticide (P_0 , P_1 and P_2) levels applied (KE+MP means, Exp.2).

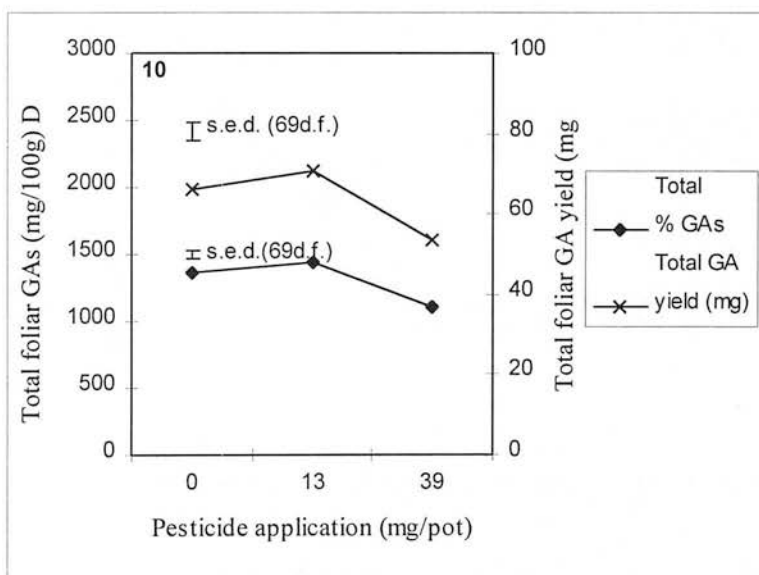


Figure 3.10. Total content (mg/100g) and total per plant (mg) foliar dry weight (DW) glycoalkaloid (GAs) production for three pesticide (P_0 , P_1 and P_2) levels applied (KE+MP means, Exp.2).

The significant ($P<0.01$) effect of the pesticide on the fresh and dry weight foliar glycoalkaloid production was mainly due to the differences observed in very low (N_1) and low (N_2) nitrogen treatments (Figures 3.11 & 3.12). No differences in glycoalkaloid production due to pesticide application were observed in medium (N_3) and high (N_4) nitrogen treatments.

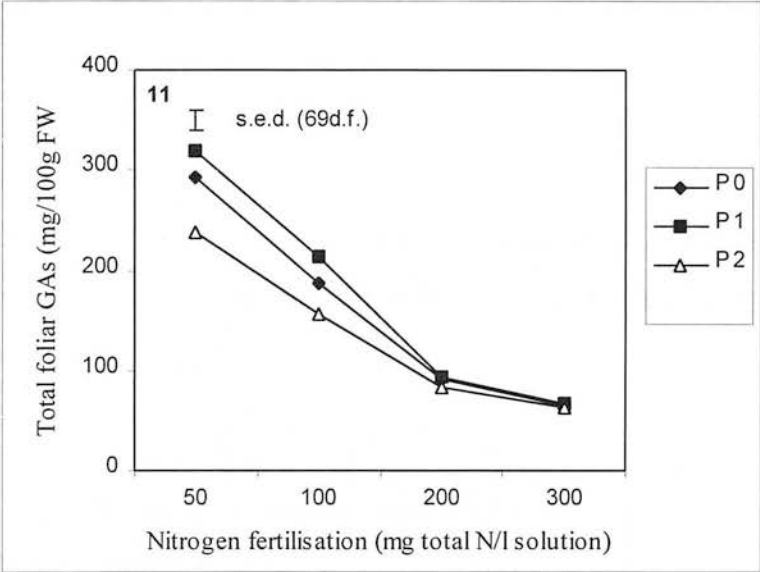


Figure 3.11. Total foliar glycoalkaloid production of both potato varieties (KE+MP) as affected by nitrogen (N) fertilisation for three pesticide levels (P_0 , P_1 and P_2) expressed in mg of glycoalkaloids per 100g of fresh weight (FW).

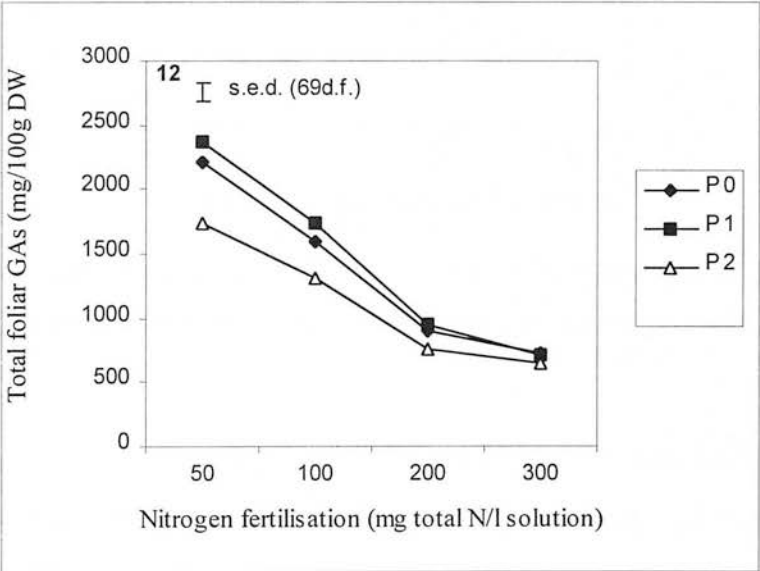


Figure 3.12. Total foliar glycoalkaloid production of both potato varieties (KE+MP) as affected by nitrogen (N) fertilisation for three pesticide levels (P_0 , P_1 and P_2) expressed in mg of glycoalkaloids per 100g of dry weight (DW).

In the very low nitrogen (N_1) treatments recommended amounts of pesticide (P_1) increased although not significantly ($P>0.05$) the amounts of glycoalkaloids produced by both cultivars (KE+MP means) compared to control (P_0). Elevated amounts (P_2) of aldicarb had the opposite effect and decreased significantly ($P<0.01$) the glycoalkaloids produced compared to control (P_0). No significant ($P>0.05$) differences were observed in the total glycoalkaloid production between P_0 and P_1 to P_2 in the low (N_2) nitrogen treatment.

King Edward

Figure 3.13 illustrates the total foliar fresh weight glycoalkaloid production as this is affected by the N fertilisation for three different pesticide levels applied to the variety King Edward.

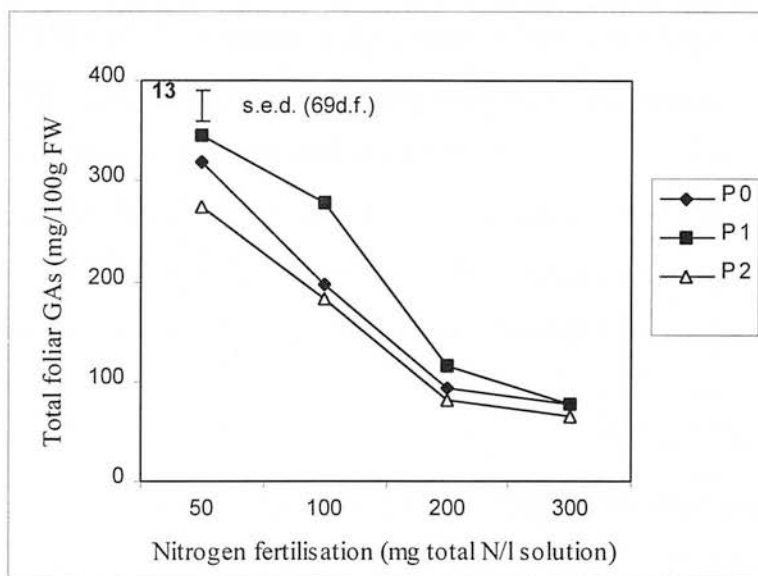


Figure 3.13. Total foliar glycoalkaloid production of potato cv. King Edward as affected by nitrogen (N) fertilisation for three pesticide levels (P_0 , P_1 and P_2) expressed in mg of glycoalkaloids per 100g of fresh weight (FW).

Plants grown in N_1P_0 treatment produced significantly ($P<0.001$) higher amounts of total glycoalkaloids compared to plants grown in N_2P_0 , N_3P_0 and N_4P_0 treatments. The differences between N_2P_0 to N_3P_0 and N_4P_0 treatments were highly significant ($P<0.001$), while no significant ($P>0.05$) differences were observed between N_3P_0 and N_4P_0 treatments. In addition, plants grown in N_1P_1 treatment produced

significantly ($P < 0.05$) higher amounts of total glycoalkaloids compared to plants grown in N_2P_1 treatment. The difference between N_1P_1 and N_2P_1 to N_3P_1 and N_4P_1 treatments was highly significant ($P < 0.001$). No significant ($P > 0.05$) differences were observed between N_3P_1 and N_4P_1 treatments.

Similar effects were observed for the N fertilisation of P_2 treatments. In particular, plants grown in N_1P_2 treatment produced significantly ($P < 0.01$) higher amounts of total glycoalkaloids compared to plants grown in N_2P_2 treatment. The difference between N_1P_2 to N_3P_2 and N_4P_2 treatments was highly significant ($P < 0.001$). A significant ($P < 0.01$) difference was observed between N_2P_2 and N_3P_2 treatments and a highly significant ($P < 0.001$) difference between N_2P_2 and N_4P_2 treatments. No significant ($P > 0.05$) differences were observed between N_3P_2 and N_4P_2 treatments.

No significant ($P > 0.05$) differences were observed between N_1P_0 to N_1P_1 and N_1P_2 treatments. The difference between N_1P_1 and N_1P_2 treatments was significant ($P < 0.05$). In addition a significant difference ($P < 0.01$) was observed between N_2P_0 to N_2P_1 and between N_2P_1 to N_2P_2 treatments. No significant ($P > 0.05$) differences were observed between N_2P_0 and N_2P_2 treatments. In N_3 and N_4 nitrogen treatments no significant ($P > 0.05$) differences were observed for the different levels of pesticide.

Maris Piper

Similar results to these of variety King Edward (Fig. 3.14) were produced for the potato variety Maris Piper. Plants grown in N_1P_0 treatment produced significantly ($P < 0.01$) higher amounts of total glycoalkaloids compared to plants grown in N_2P_0 treatment. This difference was highly significant ($P < 0.001$) for N_3P_0 and N_4P_0 treatments. The differences between N_2P_0 to N_3P_0 and N_4P_0 treatments were significant ($P < 0.01$) and highly ($P < 0.001$) significant respectively. No significant ($P > 0.05$) differences were observed between N_3P_0 and N_4P_0 treatments. In addition plants grown in N_1P_1 treatment produced significantly ($P < 0.001$) higher amounts of total glycoalkaloids compared to plants grown in N_2P_1 , N_3P_1 and N_4P_1 treatments.

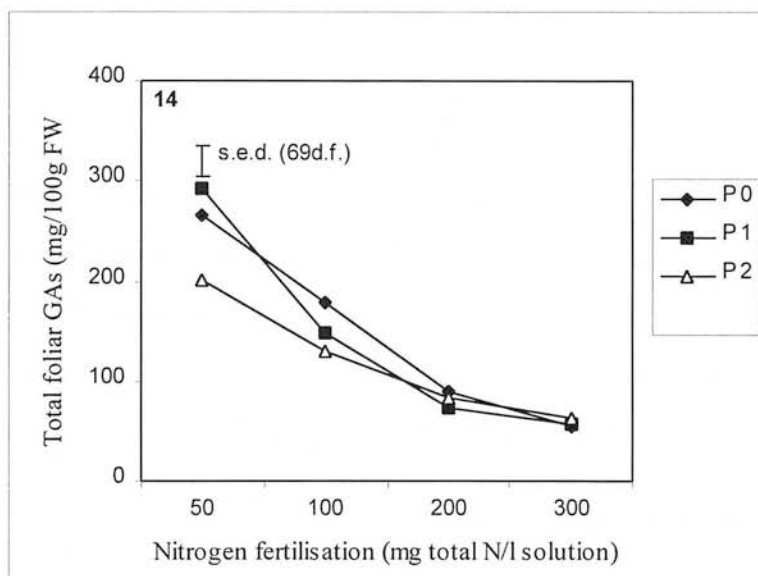


Figure 3.14. Total foliar glycoalkaloid production of potato cv. Maris Piper as affected by nitrogen (N) fertilisation for three pesticide levels (P_0 , P_1 and P_2) expressed in mg of glycoalkaloids per 100g of fresh weight (FW).

The difference between N_2P_1 to N_3P_1 treatments and that between N_2P_1 to N_4P_1 treatments were significant - ($P < 0.05$) and ($P < 0.01$) respectively. No significant ($P > 0.05$) differences were observed between N_3P_1 and N_4P_1 treatments. Similar effects were observed for the N fertilisation of P_2 treatments. In particular, plants grown in N_1P_2 treatment produced significantly ($P < 0.05$) higher amounts of total glycoalkaloids compared to plants grown in N_2P_2 treatment. The difference between N_1P_2 to N_3P_2 and N_4P_2 treatments was highly significant ($P < 0.001$). No significant ($P > 0.05$) difference was observed between N_2P_2 and N_3P_2 treatments while the difference between N_2P_2 and N_4P_2 treatments was significant ($P < 0.05$). No significant ($P > 0.05$) differences were observed between N_3P_2 and N_4P_2 treatments. No significant ($P > 0.05$) differences were observed between N_1P_0 and N_1P_1 treatments. The difference between N_1P_0 and N_1P_2 treatments was significant ($P < 0.05$) while N_1P_1 treatment produced significantly ($P < 0.01$) more total glycoalkaloids to those produced in N_1P_2 treatment. No significant ($P > 0.05$) differences were observed for any of the pesticide levels applied for the rest of the N fertilisation treatments.

3.3.7 Effects of nitrogen fertilisation and pesticide application on individual glycoalkaloids

During analysis of cv. Maris Piper samples using HPLC, a second peak was produced in addition to the one usually observed for α -solanine and to the one usually observed for α -chaconine (Appendices 3 & 4). These peaks were attributed to other forms of potato glycoalkaloids and in particular to β -solanine and β -chaconine. Table 3.9a shows the amounts of individual glycoalkaloids produced by both potato varieties as were affected by nitrogen fertilisation.

Table 3.9a. Effects of nitrogen (N) fertilisation on the individual glycoalkaloid production of potato plants cvs. King Edward and Maris Piper

Nutrient solution	King Edward		Maris Piper			
	α -sol	α -cha	α -sol	β -sol	α -cha	β -cha
N ₁	106.8	205.3	73.4	24.6	155.3	0.0
N ₂	78.8	141.1	41.9	17.6	60.9	31.7
N ₃	35.5	61.7	22.6	9.8	31.7	17.6
N ₄	25.8	47.0	14.7	8.2	18.8	16.2
SED	6.6	12.1	4.4	1.7	7.9	4.3
DF	36	36	36	36	36	27
P	***	***	***	***	***	***

SED: standard error of difference between means, DF: degrees of freedom

*** P< 0.001

N₁: 50 mg total nitrogen/l solution, N₂: 100 mg total nitrogen/l solution, N₃: 200 mg total nitrogen/l solution, N₄: 300 mg total nitrogen/l solution

α -sol: α -solanine, β -sol: β -solanine, α -cha: α -chaconine, β -cha: β -chaconine

All individual glycoalkaloid values expressed in mg/100g FW

There was a highly significant ($P<0.001$) effect of N fertilisation on the production of α -solanine and α -chaconine for both varieties examined. As it was expected from the results obtained for total glycoalkaloids highly significant ($P<0.001$) differences were observed in the amounts of α -solanine and α -chaconine produced by the potato cv. King Edward for all of the comparisons between N₁, to N₂, N₃, and N₄ treatments. The differences between to N₂, N₃, and N₄ treatments were highly significant ($P<0.001$) where no significant differences were observed between N₃, and N₄ treatments. The same results were produced after the analysis of potato cv. Maris Piper for the forms of α -solanine and α -chaconine. However no β -chaconine was detected in cv. Maris Piper plants grown in the lowest N₁ nitrogen treatment.

In addition, N fertilisation affected significantly ($P < 0.01$) the solanine to chaconine (S:C) ratio (Table 3.9b). Although α -solanine production increased significantly ($P < 0.01$) with N fertilisation from N_1 to N_2 and N_3 treatments—especially in cv. King Edward—elevated amounts of N in the nutrient solution (N_4) had the opposite effect.

Table 3.9b. Interaction effects of nitrogen (N) fertilisation x cultivar on the individual glycoalkaloid production of potato plants cvs. King Edward and Maris Piper

Nutrient solution	King Edward			Maris Piper		
	Total solanine	Total chaconine	S:C ratio	Total solanine	Total chaconine	S:C ratio
N_1	106.8	205.3	34:66	98.0	155.3	39:61
N_2	78.8	141.1	36:64	59.5	92.6	39:61
N_3	35.5	61.7	37:63	32.4	49.3	40:60
N_4	25.8	47.0	35:65	23.0	35.0	40:60
SED	6.34	10.7	0.008	6.34	10.7	0.008
DF	69	69	69	69	69	69
P	**	**	**	**	**	**

SED: standard error of difference between means, DF: degrees of freedom

** $P < 0.01$

N_1 : 50 mg total nitrogen/l solution, N_2 : 100 mg total nitrogen/l solution, N_3 : 200 mg total nitrogen/l solution, N_4 : 300 mg total nitrogen/l solution

All individual glycoalkaloid values expressed in mg/100g FW

S:C: percent ratio of total solanine to total chaconine

In cv Maris Piper the solanine to chaconine distribution was different between low (N_1 - N_2) and medium to high (N_3 - N_4) N levels; significantly ($P < 0.05$) less solanine was produced in the low N (N_1 - N_2) group. In general cv. Maris Piper produced significantly less solanine ($P < 0.01$) and chaconine ($P < 0.001$) compared to that produced by cv. King Edward (Table 3.9c).

Table 3.9c. Effects of cultivar on the individual glycoalkaloid production of potato plants cvs. King Edward and Maris Piper

	King Edward			Maris Piper		
	Total solanine	Total chaconine	S:C ratio	Total solanine	Total chaconine	S:C ratio
	61.7	113.7	35.5:64.5	53.2	83.0	39.5:60.5
SED	3.2	5.4	0.004	3.2	5.3	0.004
DF	69	69	69	69	69	69
P	**	***	***	**	***	***

SED: standard error of difference between means, DF: degrees of freedom

*** $P < 0.001$, ** $P < 0.01$

S:C: ratio of total solanine to total chaconine

However the proportion of solanine to chaconine (S:C ratio) in cv. King Edward was significantly lower ($P < 0.001$) compared to cv. Maris Piper. Table 3.10a shows the amounts of individual glycoalkaloids produced by both potato varieties as these were affected by pesticide application.

Table 3.10a. Effects of pesticide application on the individual glycoalkaloid production of potato plants cvs. King Edward and Maris Piper

Pesticide applied	King Edward		Maris Piper			
	α -sol	α -cha	α -sol	β -sol	α -cha	β -cha
P ₀	60.7	111.0	41.6	16.5	72.4	16.4
P ₁	71.9	132.0	39.7	15.7	70.8	16.5
P ₂	52.6	98.3	33.2	13.0	56.8	16.1
SED	5.8	10.5	3.8	1.5	6.9	4.3
DF	36	36	36	36	36	27
P	**	**	NS	NS	NS	NS

SED: standard error of difference between means, DF: degrees of freedom

** $P < 0.01$, NS non significant

P₀: 0 mg aldicarb / pot, P₁: 0.13 mg aldicarb / pot, P₂: 0.39 mg aldicarb / pot

α -sol: α -solanine, β -sol: β -solanine, α -cha: α -chaconine, β -cha: β -chaconine

Recommended amounts of pesticide P₁ applied in potato cv. King Edward, increased –although non-significantly ($P > 0.05$)– the amounts of α -solanine and α -chaconine produced, compared to control (P₀). Elevated amounts of pesticide P₂ had an opposite but no significant ($P > 0.05$) effect.

Table 3.10b. Interaction effects of pesticide application x cultivar on the individual glycoalkaloid production of potato plants cvs. King Edward and Maris Piper

Pesticide applied	King Edward			Maris Piper		
	Total solanine	Total chaconine	S:C ratio	Total solanine	Total chaconine	S:C ratio
P ₀	60.7	111	36:64	58.1	88.8	40:60
P ₁	71.9	132	36:64	55.4	87.3	39:61
P ₂	52.6	98.3	35:65	46.1	72.9	39:61
SED	5.5	4.9	0.007	5.5	4.9	0.007
DF	69	69	69	69	69	69
P	NS	NS	NS	NS	NS	NS

SED: standard error of difference between means, DF: degrees of freedom

NS non significant

P₀: 0 mg aldicarb / pot, P₁: 0.13 mg aldicarb / pot, P₂: 0.39 mg aldicarb / pot

S:C: ratio of total solanine to total chaconine

The significant effect observed ($P < 0.01$) was due to elevated amounts of α -solanine and α -chaconine produced to P_1 treatments compared to these produced in P_2 treatments (Table 3.10a). However no significant differences ($P > 0.05$) were observed for the individual glycoalkaloids produced by potato cv. Maris Piper. Non significant ($P > 0.05$) differences were observed in the amounts of total solanine and total chaconine on the individual glycoalkaloid production and the α -solanine to α -chaconine ratio between the two cultivars due to pesticide application (Table 3.10b). The amounts of α -solanine were reduced in the P_2 treatments in potato cv. King Edward.

3.4 Discussion

Plants from both experiments grown in low nitrogen treatments, produced higher amounts of total and individual glycoalkaloids compared to plants grown in high nitrogen treatments. These data are in agreement with the results reported by (Nowacki *et al.*, 1975). The declining glycoalkaloid content of potato foliage with the simultaneous increase of nitrogen fertilisation, was attributed to the limited production of soluble carbohydrates and acetate, which are considered as the glycoalkaloid synthesis precursors (Guseva *et al.*, 1960) as this has been stated in general introduction of this thesis; elevated amounts of nitrogen were enhancing the accumulation of glutamate and restrained the carbohydrate synthesis leading to a reduction of the amounts of glycoalkaloids finally produced.

The results from both experiments suggest a gradual reduction of glycoalkaloid production combined with the increase of nitrogen in the nutrient solutions. This was also observed in both varieties for all pesticide levels applied. An exception was the N_2P_1 treatment of exp. 1 where the glycoalkaloids detected were higher (although non-significantly) compared to the N_1P_1 treatment (Figures 3.5 & 3.6). The highest level of glycoalkaloids produced (636.5 mg/100g FW) was observed in the lowest nitrogen – no pesticide (N_1P_0) treatment in exp. 1 (Figures 3.5 & 3.6). In general the glycoalkaloid levels recorded in the nitrogen deficient plants were far higher than the

range of 30-160 mg/100g FW, which is considered typical for potato leaves (Dao & Friedman, 1996). In addition nitrogen fertilisation had a highly significant ($P < 0.001$) effect on the individual glycoalkaloid production (Tables 3.3b & 3.9a). This was expected as individual glycoalkaloids compose part of the total (the ratio of α -solanine to α -chaconine is approximately 1:1.5 (Guseva *et al.*, 1960).

Since nitrogen fertilisation induced a concurrent increase of the green matter, it could be suggested that the glycoalkaloid reduction observed is the result of a dilution effect emerging from the incorporation of an amount of glycoalkaloids in a constantly increasing quantity of fresh or dry tissue. This is supported by the fact that although in the second experiment, plants of all treatments were harvested in the same physiological stage (prior to flowering), plants in low nitrogen treatments (N_1 and N_2) had produced less green matter compared to plants grown under medium or high nitrogen treatments (N_3 and N_4). Due to a higher yield of green matter on higher fertilised plants the total foliar glycoalkaloid yield was increased with the nitrogen fertilisation level. This was observed in cv. King Edward in both experiments as well as in cv. Maris Piper in exp. 2. The peak of the total glycoalkaloid yield observed in the N_2 treatment in exp. 2 (Figures 3.7 & 3.8) was mainly due to the elevated glycoalkaloid yield produced in cv. King Edward (Table 3.6b) and could possibly be explained by the elevated amounts of glycoalkaloids observed in the N_2P_1 treatment (Fig. 3.13). By contrast the total glycoalkaloid yield produced in cv. Maris Piper follows a gradual and constant increase with nitrogen fertilisation (Table 3.6b).

In general both cultivars showed similar response to nitrogen fertilisation with cv. Maris Piper producing less total glycoalkaloids compared to those produced by cv. King Edward. The total glycoalkaloid values which were produced in potato leaves of cv. King Edward showed a slight increase in exp. 1, compared to those produced by the same cultivar in exp. 2, for each of the four nitrogen treatments applied. This could be explained by the fact that the amounts of green matter produced by cv. King Edward in exp. 1 were reduced compared to that produced in exp. 2. Another difference was observed in the proportion of α -solanine to α -chaconine. The potato

cv. King Edward produced more α -chaconine compared to that produced by the cv. Maris Piper. Nitrogen fertilisation increased the amounts of α -solanine produced for both varieties. An exception was observed in the highest nitrogen fertilisation treatments applied to cv. King Edward where the amounts of α -solanine decreased. The α -solanine increase however was not observed in cv King Edward in exp. 1.

The results reported here are in contrast with those obtained by Ahmed & Müller (1979), for leaves, stems and tubers and by Mondy & Munshi (1990), Cronk *et al.* (1974) and Love *et al.* (1994), who reported significant increases in the total tuber concentrations of glycoalkaloids by increasing nitrogen fertilisation of potato cultivars. Love *et al.* (1994), suggested that since glycoalkaloids are nitrogen-containing molecules any nitrogen deficiency would be expected to influence the formation of such compounds by reducing the amounts produced. By contrast Waller & Nowacki (1978), suggested that all plants do not react to increased nitrogen fertilisation by increasing alkaloid biosynthesis. While the *Nicotiana*, *Lupinus* and *Hordeum* species produce two- to tenfold increases of alkaloid content, this does not happen with plants producing indole alkaloids and *Solanum* glycoalkaloids.

Nowacki *et al.* (1975), suggested that the depressed content of glycoalkaloids observed in potato foliage could be elucidated as follows: due to a higher level of available nitrogen the plant uses all sugars and nitrogenless compounds like acetate for synthesis of acceptors for nitrogen e.g. aspartic and glutamic acid; hence is depleted of substrates for alkaloid synthesis. Additional studies measuring the concentrations of soluble sugars as well as the soluble nitrogen forms (nitrate nitrogen, amino acids) in leaf tissues are essential to indicate possible correlation with glycoalkaloid levels. According to Mondy & Munshi (1990), application of nitrogen fertiliser may have also increased the amino acid fraction of the nitrogenous constituents in the potato tuber. The amino acids leucine, alanine (Jadhav *et al.*, 1973) and arginine (Kameko *et al.*, 1976) have been shown to be incorporated in the formation of the nitrogen-containing skeleton of the aglycon solanidine in glycoalkaloid synthesis. Solanidine biosynthesis may be connected with protein

metabolism (Han *et al.*, 1989). Increases in the amounts of these amino acids may have increased glycoalkaloid synthesis. Nair *et al.* (1981), suggested that chlorophyll synthesis and photosynthetic activity of the chloroplast in a potato tuber are essential for solanine synthesis. Nitrogen fertilisation could increase the chloroplast content of the plant through increased leaf growth and the entire enzyme machinery for solanidine synthesis from CO₂ is located in chloroplasts (Nair *et al.*, 1981). Answers to these questions could be produced when additional research has been conducted on the possible effects of nitrogen fertilisation on the enzymes involved in glycoalkaloid synthesis.

In exp. 1, the glycoalkaloids produced in cv. King Edward in the control treatment (no pesticide - P₀) were significantly higher ($P < 0.01$) compared to those produced in the P₁ and P₂ treatments. By contrast in exp. 2 for the same cultivar, significantly more glycoalkaloids ($P < 0.05$) were produced in P₁ treatments compared to control, while no differences were observed between control and the highest (P₂) pesticide treatment. In exp. 2 the main overall effect of pesticide observed was mainly due to the differences observed in potato cv. King Edward since no differences were observed in cv. Maris Piper. Hence the two cultivars reacted in a different way to pesticide application. Although these responses are somewhat difficult to interpret, they might be explained by the fact that plants grown in exp. 1 were receiving different amounts of nutrient solutions within a nitrogen treatment. This could have altered the total amount of nitrogen added, changed the growing rates of the plants and consequently the amounts of glycoalkaloids produced. Since plants in exp. 1 were at different developmental stages when harvested, while plants in exp. 2 were not, differential plant development might have contributed to the discrepancy observed between the two experiments. Another plausible explanation can be possible fluctuations of the concentration of the glycoalkaloids, since it is known (Robinson, 1974) that alkaloids in general are considered as substances the concentrations of which can fluctuate during a single day.

In the first experiment the ventilation system of the plant growth cabinets was not

circulating the air uniformly and as a result pot trays located on the right side of the cabinets needed watering more often compared to trays located in the centre or the left side. Random relocation of the plants was applied daily concurrently with tray watering to help moderate a possible bias on the results. The total amounts of added solutions were not measured because the small size of the cabinets increased the difficulties of taking measurements; so practically the actual amounts of N added are not known. In addition, potato plants were harvested on the same day but due to the different nitrogen treatments were at different physiological stages. In particular, plants grown in the highest nitrogen treatment had reached the stage of appearance of the first buds while plants grown in the low nitrogen treatment were still at early growth stages. Leaves and stems were harvested and placed in the freeze without being immersed previously in liquid nitrogen. This could have induced further enzymatic activity which could have altered the soluble nitrogen (nitrate, amino acid) as well as glycoalkaloid composition of the sampled tissues. The encouraging results obtained from the first experiment combined with the previously mentioned imperfections suggested the repeat of the experiment in a plant growth room using a second potato variety. In the second experiment all potato plants were collected in the same physiological stage (appearance of first buds-before flowering) but in different dates. Hence the N_4 and the N_1 treatments were collected first and last respectively.

There is limited literature dealing with foliar glycoalkaloid analysis using HPLC. More recent are the works by Friedman & Dao (1992) and Dao & Friedman (1996), who suggested that due to large variations in both α -solanine and α -chaconine contents of fresh leaves, foliar glycoalkaloids should be determined from freeze-dried rather than fresh leaves. According to Percival (1993), from all available methods for glycoalkaloid analysis available to quantify glycoalkaloids (colourimetric, titrimetric, thin layer chromatographic, gas chromatographic and high performance liquid chromatographic (HPLC)), the latter is the most applicable for these metabolites. It is considered to be a fast, accurate and reproducible method for determining individual glycoalkaloid concentrations. Hellenas (1986), reported that

this procedure for glycoalkaloid determination is in agreement with other methods such as an improved ELISA technique and a colourimetric procedure. However potato foliage glycoalkaloid analyses by using the technique developed by Hellenas (1986), have not been reported so far. The described extraction-HPLC procedure required only a very small amount of material (0.05g).

The composition of the mobile phase used for HPLC was important in ensuring full separation of α -solanine and α -chaconine. The appearance of the non-expected secondary peaks which were attributed to β -solanine and β -chaconine in cv. Maris Piper would have not been achieved if the initial proportion of acetonitrile/water (35:65) in the mobile phase had been preserved. Short trials altering the acetonitrile to water volumes proved that the 34:66 (vol/vol) acetonitrile/water is the optimum proportion suitable for plotting a second peak during the analysis of cv. Maris Piper samples. In addition the method and standards used for the calculation of the β -forms, was the same used for the calculation of α - forms. It is recognised that the values measured are subjected to errors but currently standards of the β -forms are not available. Isolation of β - and γ - forms has been achieved from incomplete hydrolysis mixtures of the parent compounds (α - forms) and characterized by HPLC and mass spectrometry (Friedman *et al.*, 1993). Although the separation of β -forms from α - forms of solanine and chaconine was not perfect in most of the cases, the chromatograms had good baselines, showed no interference and were reproducible. The presence of β -forms of solanine and chaconine in potato cv, Maris Piper indicates that either these forms are not present in the foliage of potato cv. King Edward or the method developed is not sufficient for the separation of β -forms in cv. King Edward. However β -solanine and β -chaconine, have not been detected in the potato foliage so far. Further studies should concentrate on improving the HPLC analytical technique and achieving better separation of β -forms of solanine and chaconine in potato foliage samples.

Glycoalkaloids are considered as secondary metabolites accumulating in fast growing tissues, or regions showing high metabolic activity. Meristematic tissues like eye

regions or tip sprouts are active sites of glycoalkaloid biosynthesis (Han *et al.*, 1989). Thus one would expect that factors which increase metabolism or growth rates like nitrogen fertilisation might also enhance glycoalkaloid production. However increased nitrogen fertilisation resulted in significant ($P < 0.001$) reduction of the total and individual glycoalkaloid content. These results in relation to those observed in the previous chapter give rise to the concept that excessive nitrogen fertilisation may induce an adverse effect on the defensive system of the potato plant. This effect may come about by an increase in the food quality of the phloem sap –which is regarded to be the main feeding site of the aphids- and/or by a reduction of the concentration of total glycoalkaloids or an alteration in the proportion of individual glycoalkaloids (α -solanine to α -chaconine ratio). In conclusion it could be implied that in organic agriculture where the amounts of available nitrogen are limited, a crop could possibly be characterised as of a better health standard due to the presence of naturally occurring defensive allelochemicals in such concentrations able to deter insect infestations. In conventional agriculture where nitrogen fertilisation is usually in surplus, the need for a more reasonable use could be addressed. Experiments in which aphid-infested potato plants grown in low and high nitrogen solutions will be assessed for glycoalkaloid concentrations may provide valuable information regarding pest-induced defence.

Chapter 4

**Effects of aphid infestation on the foliar
glycoalkaloid production of potato,
grown in protected environment**

4.1 Introduction

Secondary metabolites are often considered to be chemicals produced by a plant as a defence mechanism or a reaction to stress induced by a pathogen or a herbivore. Phytochemists consider stress as an external constraint that limits the normal production of secondary metabolites in plants (Timmermann & Steelink, 1998). It has been reported (Young, 1991) that in many cases the toxin content of damaged plant tissue appears to be higher than that in undamaged tissue, the difference being attributed to pest stress. For example pea aphids *A. pisum* (Harris) feeding on alfalfa (*Medicago sativa* L.) stimulate the biosynthesis of coumestrol –an isoflavonoid having oestrogenic activity on mammals- which makes the plants more resistant to subsequent aphid infestation (Loper, 1968). In addition root damage to *Brassica* species by insect pests caused increased concentrations of toxic glucosinolates in the foliage (Birch *et al.*, 1992).

Almost any stress that affects growth and development of the potato crop can also have some effect on the glycoalkaloid content of potato tubers. Most research aimed at glycoalkaloids has concentrated on the effects of environmental and physical changes that occur during the growth, harvesting, storage and processing of potato tubers. In addition, there is an extensive literature covering the effects of various stress factors such as mechanisation of harvest and post-harvest handling (Olsson, 1989; Mondy *et al.*, 1987; Ahmed & Müller, 1978; Wu & Salunkhe, 1976; Salunkhe *et al.*, 1972) or fungal infection (Deahl *et al.*, 1973) in connection with glycoalkaloid concentrations of the potato tubers.

It has been suggested that in potato plants the concentration of α -solanine and α -chaconine in tubers may increase when plants are under stress caused by fungal pathogens (Gull & Isenberg, 1960). In particular Locci & Kuc (1967), supported the assumption that the accumulation of glycoalkaloids in potato tubers is a response to physiological stress caused by mechanical injury, or by the interaction between the host and the infesting microorganism. However Frank *et al.* (1975), concluded that

the glycoalkaloid levels show no pattern for decrease or increase immediately after infection with any fungal pathogen. Glycoalkaloids had no direct relationship to resistance against early blight (*Alternaria solani*), late blight (*Phytophthora infestans*), common scab (*Streptomyces scabies*), and Verticillium wilt (*Verticillium albo-atrum*). According to Deahl *et al.* (1973), the tuber glycoalkaloid contents from late blight inoculated potato plants were about the same as those of tubers from healthy (fungicide-protected) plants for both resistant and susceptible clones. So blight infection did not appear to cause higher tuber glycoalkaloid contents.

There is a limited number of studies that have specifically examined the effects of pest-related biological stress on glycoalkaloid contents of potatoes. In field and growth room studies Hlywka *et al.* (1994), indicated that the tuber glycoalkaloid concentrations of potatoes subjected to defoliation damage by Colorado potato beetles, *L. decemlineata*, were consistently greater than those concentrations found in tubers from undamaged plants. These insects exhibit a preference for the tender young tissue although it contains higher concentrations of glycoalkaloids. By contrast, the same authors reported that damage to plants by potato leafhoppers *E. fabae* did not have any apparent effect on tuber glycoalkaloid contents. This could possibly be related to the type of stress resulting from feeding on the plant by each of these insects. Colorado potato beetles are very destructive and aggressive chewing insect pests, whereas potato leafhoppers are phloem feeders. However in *Solanum americanum* Mill., the total glycoalkaloid content in the stalk, leaves and especially the fruits was always lower in plants infested with the aphid *Aphis fabae* ssp. *solanella*, compared with non-infested plants (Zullo *et al.*, 1984). In this work, it was not possible to determine whether this low level was a result of the infestation or whether the reduced content of glycoalkaloids had made it possible for the infestation to develop. The objective of these experiments is to determine the foliar glycoalkaloid concentrations in potato plants grown under different nitrogen fertilisation levels and subjected to extensive damage by the peach potato aphid *M. persicae* during their early stages of growth.

4.2 Materials and Methods

Two experiments were conducted in the plant growth unit of the SAC. In the first experiment (exp. 1), the potato variety King Edward was grown in the glasshouse between May and July 1995. In the second experiment (exp. 2), the potato varieties King Edward and Maris Piper were grown in a plant growth room between May and July 1996. In both experiments, potato plants were infested by the aphid *M. persicae*. The glycoalkaloids produced by the aphid-infested plants were compared to those produced by aphid-free plants. The same growing media and nutrient solutions were used in both experiments.

4.2.1 Growing media

The potato plants were grown in the 'semi-hydroponic' culture system already described in Chapter 3. Perlite was used as a plant growing substrate and the nutrient solutions contained different amounts of nitrogen in both ammonium (NH_4^+) and nitrate (NO_3^-) forms.

4.2.2 Plant material

The same plant material as that described in Chapter 3 was used for both experiments. Pots with developing potato sprouts were placed in a glasshouse (experiment 1) and in a plant growth room (experiment 2).

4.2.3 Nutrient solution and aphid infestation

Glasshouse experiment (exp. 1)

Four different nitrogen treatments were combined with aphid-infested and uninfested plants. The potato plants (cv. King Edward) were divided in to five groups representing aphid-infested and non-infested plants harvested on different dates. Four replicates were used per treatment. The nitrogen (N) treatments and the way of application were the same (N_1 , N_2 , N_3 , and N_4 representing 50, 100, 200 and 300 mg total N/lit respectively) as previously described under Materials and Methods in Chapter 3, for each one of the five groups. The total amounts of nitrogen added in

each group were 150, 200, 400 and 600 mg /tray. Equal volumes of distilled water were added to all replicates to reduce condensation of nutrient solutions owing to water evaporation and leading to possible toxic effects of nitrogen. In all the feeds, the NO_3^- to NH_4^+ ratio was held the same at 4:1.

The aphids intended to infest the potato plant were derived from the clone lineage culture of the aphid *M. persicae*, which was established in the insectory (Chapter 2). Thirty standardised adult aphids per plant (approximately five/leaf) were transferred from the basic culture (Chinese cabbage) to the treatments subjected to infestation. Plants were infested with aphids when they had reached a predetermined developmental stage (full development of the second true leaf). Due to differences in nitrogen fertilisation, N_3 and N_4 , plants reached this stage faster compared to plants grown under lower nitrogen treatments (N_1 , and N_2); hence they were infested first with the aphids. In particular the five different groups of aphid-infested and non-infested plants were:

Group 1, (G1): Non aphid-infested plants harvested after 15 days from the effective starting date of the experiment (considered to be the first watering with nutrient solutions).

Group 2, (G2): Aphid-infested plants after 15 days from the effective starting date of the experiment and harvested after 30 days from infestation.

Group 3, (G3): Non aphid-infested plants harvested after 45 days from the effective starting date of the experiment.

Group 4, (G4): Aphid-infested plants after 15 days from the effective starting date of the experiment, aphids killed after 30 days from infestation and plants harvested after 15 days from removal of aphid infestation.

Group 5, (G5): Non aphid-infested plants harvested after 60 days from the effective starting date of the experiment.

A schematic representation of the aphid infestation treatments applied to the potato plants and times of harvesting is given in graphic representation 4.1:

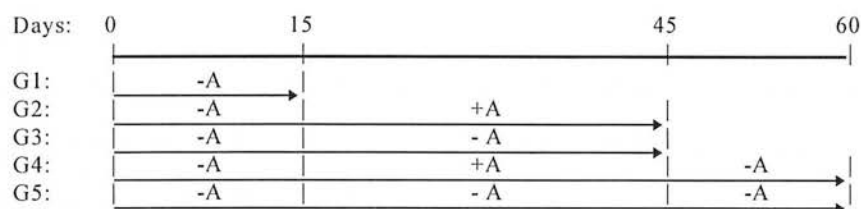


Figure 4.1. Schematic representation of the *Myzus persicae* infestation groups (G1, G2, G3, G4 and G5) applied to the potato plants plotted against times of harvesting; +A: aphid-infested period, -A: aphid-free period.

Pots were randomly relocated in the glasshouse every 48h. Both aphid-infested and non-infested plants were covered with fine nylon mesh hoods for isolation and prevention of aphid cross-infestations. The extermination of the aphids in the aphid-infested G4 plants was achieved by immersing the whole plant in a plastic tank containing a soft soap solution 0.5 %. The same treatment was applied to G5 (non aphid-infested) group. From a statistical viewpoint exp. 1 was a replicated two factor experiment with nitrogen applied at four levels (N_1 , N_2 , N_3 , and N_4) to 5 levels of aphid/harvest plants (G1, G2, G3, G4 and G5). The treatment combinations were repeated four times. In total 80 pots were placed in the glasshouse. (exp. 1). The data were analysed using the Analysis of Variance procedure of MINITAB® (vs.11.1). The output of analysis of variance, was tables of means with standard errors. The differences between means were measured by t-test.

Plant growth room experiment (exp. 2)

Two different nitrogen treatments were combined with aphid-infested and non-infested plants. The potato plants (cvs. King Edward and Maris Piper) were divided as in exp. 1 in five groups representing aphid-infested and non-infested plants harvested at different dates. By contrast to the previous experiment, the nitrogen (N) treatments were limited to medium and very high levels (N_2 , N_4 representing 100, and 300 mg total N/lit respectively). The application method was the same as previously described (under Materials and Methods in Chapter 3) for each of the five groups. The total amounts of nitrogen added in each group were 200, and 600 mg /tray.

Aphid infestation was achieved as in exp. 1 and plants were infested when they approached the same (as in exp. 1) developmental stage (full development of the second true leaf). The five different groups of aphid-infested and non-infested plants have been previously described (exp. 1). Sample preparation, extraction, purification and HPLC analysis were performed as previously described in Chapter 3. Exp. 2 was a replicated three factor experiment with nitrogen applied at two levels (N_2 , and N_4) to 5 levels of aphid/harvest plants (G1, G2, G3, G4 and G5) of potato cultivars King Edward and Maris Piper. The treatment combinations were repeated four times. In total, 80 pots were placed in the plant growth room at 20°C, 50% RH, and long day (16:8) conditions, in two cages (4.5m x 1m x 1m) made of nylon mesh isolating the infested from the non-infested plants. Pots were randomly relocated in the cages every second day. The data were analysed using the Analysis of Variance procedure of MINITAB® (vs.11.1). The output of analysis of variance, was tables of means with standard errors. The differences between means were measured by t-test.

4.2.4 Assessment of aphid performance

In experiment 1 the aphid performance was measured by using the methods of individual adult cumulative fecundity and that of the intrinsic rate of natural increase as both have been described in the artificial diet experiments of chapter 2. These measurements started simultaneously with the infestation and continued throughout the period of aphid infestation. For this reason, the aphids were confined to leaves with leaf clip-on cages which were constructed as described by Adams & van Emden (1972) and were supported by a metal wire stand. The inside edges of the cages (which were in contact with the leaf surface) were separated by 2 mm of foam to minimise damage to the potato leaves. For the measuring of the individual adult cumulative fecundity two standardised adult aphids as pre-reproductive apterae were caged on the first true leaf to reproduce. The cage was placed in the abaxial surface of the leaf. The adults and their clip cage were transferred every six days to a younger leaf to minimise damage to plants and possible detrimental effect on aphid fecundity (Dewar, 1977). Offspring were counted and removed during the morning of the first day of reproduction and then every second day thereafter for a total period

of 12 days.

For the measuring of the intrinsic rate of natural increase two standardised apterous pre-reproductive adult aphids were caged on the second true leaf to reproduce for 24 hours. The aphids were placed on the abaxial surface of the leaf. After this period the adults were removed and the 3-5 new born nymphs were monitored daily. The nymphs were left to approach the 4th instar stage and finally two were left in the clip cage to develop into apterous pre-reproductive adults; the others were discarded. As previously with the measuring of the individual adult cumulative fecundity, the pre-reproductive adults and their clip cage were transferred to a younger leaf to minimise damage to plants. This transfer was repeated every 6 days after the retained aphids reached maturity. The time taken to mature and the number of offspring produced were recorded. Offspring were counted and removed during the morning of the first day of reproduction and every second day thereafter for a total period equal to the number of days taken to develop from 1st instar to reproductive adult. The intrinsic rate of natural increase (r_m) was then calculated for each clip-cage of aphids as described in Chapter 2 according to the formula given by Wyatt & White (1977):

$$r_m = 0.74((\ln M_d)/d)$$

where d is the pre-reproductive time in days (from birth to first reproduction), and M_d is the number of progeny produced in the ensuing period of length d . The aim of the experiments presented in this chapter was to calculate the levels of the glycoalkaloids produced by plants that suffered heavy aphid infestation, and compare them to those produced by non-infested plants. To achieve this target a dense colonisation of the plants by aphids was required. For this reason the aphids were allowed to colonise the potato plants for a total period of one month; it was subsets of this population which were clip-caged. However this practice introduced difficulties with the measurement of aphid performance using the methods described earlier. In particular, the dense colonisation of the plants by the aphids prevented the essential manipulations of removing and placing the clip-cages, measuring and discarding offspring and transferring clip-cages to younger leaves undamaged by aphids. This

situation very often led to accidental damage of the caged aphids and escaping from the caging conditions due to the alarm pheromones excreted by damaged aphids adjacent to the clip-cage. In addition the removal and relocation of the mesh hoods which prevented aphid cross-infestations between aphid-infested and non-infested plants were occasionally damaging the potato leaves. The damaged leaves were excised, placed in labeled paper envelopes and stored in the deep freeze (-80°C) for further glycoalkaloid analysis. For these reasons the results obtained from these measurements are interpreted more cautiously than those obtained from the second experiment.

For the reasons described above in the second (plant growth room) experiment, a different way of measuring the aphid performance was applied: at the day of harvesting of the plant groups G2 and G3 (30 days after aphid infestation) an upper, a middle and a lower leaf from each plant were excised. The aphids (adults and nymphs) present on each leaf were counted using a hand-counter. After the measurement the aphids were brushed away and the leaf area was measured using a LI-COR leaf area meter model 3100 (Lambda Instruments Corporation, Lincoln, Nebraska, USA). The aphid abundance was calculated as the average of the aphids measured on the three leaves per cm². The same method was applied for potato plants of the G4 group. Each excised leaf of this group was placed after the aphid measurement in a labeled paper envelope and stored in the deep freeze for further glycoalkaloid analysis. For the same purpose three leaves –an upper, a middle and a lower one- from the non-aphid-infested plants of the G5 group were also excised and placed in labeled paper envelopes and stored in the deep freeze.

4.3 Results

Experiment 1

4.3.1 Effects of nitrogen fertilisation and aphid infestation on green and dry matter

Table 4.1a shows the overall effects of nitrogen fertilisation on the green matter (FW)

and dry matter (DW/FW) produced by aphid-infested and non-infested potato plants. Nitrogen fertilisation induced a highly significant ($P<0.001$) overall increase in the total amount of green matter produced. Highly significant ($P<0.001$) differences were observed between all of the four nitrogen solutions applied (Table 4.1a). In addition nitrogen fertilisation induced a highly significant ($P<0.001$) overall decrease in the total amounts of dry matter produced. In particular highly significant ($P<0.001$) differences were observed in the dry matter between N_1 and N_2 to N_3 and N_4 treatments.

Table 4.1a. Effects of nitrogen (N) fertilisation on the green (FW) and dry matter (DW/FW) produced by potato plants cv. King Edward

Nutrient solutions	Green matter FW (g)	Dry matter (DW/FW)
N_1	15.61	0.131
N_2	23.77	0.115
N_3	38.51	0.105
N_4	49.62	0.101
SED	1.4	0.004
DF	60	60
P	***	***

SED: standard error of difference between means, DF: degrees of freedom
 *** $P<0.001$
 N_1 :50 mg total nitrogen/l solution, N_2 : 100 mg total nitrogen/l solution, N_3 : 200 mg total nitrogen/l solution, N_4 : 300 mg total nitrogen/l solution
 FW: fresh weight, DW: dry weight

Aphid infestation induced an overall highly significant ($P<0.001$) decrease in the amounts of green matter produced (Table 4.1b). Aphid-infested plants (G2 & G4) produced significantly ($P<0.001$) less amounts of green matter compared to non-infested plants (G3 & G5). In addition time of harvesting did not have any significant ($P>0.05$) effect - with an exception observed in G1 -on the amounts of green matter produced. In particular no significant ($P>0.05$) differences were observed in the green matter produced between G2 and G4 treatments as well as between G3 and G5 treatments. No significant ($P>0.05$) differences were observed in the dry matter (dry to fresh weight ratio) produced between aphid-infested (G2 & G4) and non-infested plants (G3 & G5). So it seems that aphid infestation did not affect the dry matter produced. By contrast, time of harvesting affected the dry matter. In particular a significant difference was observed between G2 and G4 plants

($P < 0.01$) (both aphid-infested) and G3 and G5 plants ($P < 0.001$) (both non-infested).

Table 4.1b. Effects of aphid infestation on the green (FW) and dry matter (DW/FW) produced by aphid infested (G2, G4) and non-infested (G1, G3, G5) potato plants cv. King Edward

Green matter FW (g)					Dry matter (DW/FW)				
G1	G2	G3	G4	G5	G1	G2	G3	G4	G5
7.5	32.9	41.9	34.6	42.4	0.085	0.110	0.115	0.125	0.135
SED	1.57				0.005				
DF	60				60				
P	***				***				

SED: standard error of difference between means, DF: degrees of freedom

*** $P < 0.001$

Days:	0	15	45	60
G1:	----- ----- ----- -----			
G2:	----- ----- ----- -----			
G3:	----- ----- ----- -----			
G4:	----- ----- ----- -----			
G5:	----- ----- ----- -----			

FW: fresh weight, DW: dry weight

Table 4.1c shows the interaction effects of nitrogen fertilisation and aphid infestation on the green and dry matter produced by the potato cv. King Edward. No significant ($P > 0.05$) differences were observed in the green matter produced within the different nitrogen treatments applied in G1 group of plants which were harvested approximately 15 days from the effective starting date of the experiment (Table 4.1c).

Although the plants harvested in G1 treatment were subjected to different nitrogen solutions, they were harvested at the same developmental stage (full development of the second true leaf). Hence there were no significant ($P > 0.05$) differences in the amounts of the green matter produced. By contrast plants harvested either at 45 (G2 & G3) or at 60 (G4 & G5) days after the beginning of the experiment, showed significant ($P < 0.05$) to highly significant ($P < 0.001$) differences in the green matter produced between the different N levels applied (Table 4.1c). Highly significant ($P < 0.001$) differences in the green matter produced were observed between aphid-infested (G2 & G4) and non-infested (G3 & G5) plants. In particular aphid infestation induced a highly significant ($P < 0.001$) reduction in the amounts of green

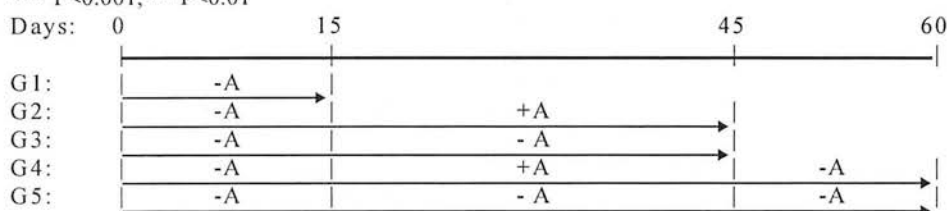
matter produced by plants grown in the N₃ treatment of the G2 and G3 groups.

Table 4.1c. Interaction effects of nitrogen (N) fertilisation x aphid infestation on the green (FW) and dry matter (DW/FW) produced by aphid infested (G2, G4) and non-infested (G1, G3, G5) potato plants cv. King Edward

Nutrient solutions	Green matter FW (g)					Dry matter (DW/FW)				
	G1	G2	G3	G4	G5	G1	G2	G3	G4	G5
N ₁	6.2	18.0	23.5	13.7	16.7	0.093	0.132	0.161	0.128	0.142
N ₂	8.7	25.3	30.0	25.7	29.1	0.091	0.115	0.104	0.123	0.143
N ₃	7.8	37.3	52.5	42.9	52.1	0.082	0.097	0.098	0.131	0.121
N ₄	7.3	51.2	61.7	56.2	71.8	0.073	0.091	0.094	0.117	0.132
SED			3.13					0.01		
DF			60					60		
P			***					**		

SED: standard error of difference between means, DF: degrees of freedom

*** P<0.001, ** P<0.01



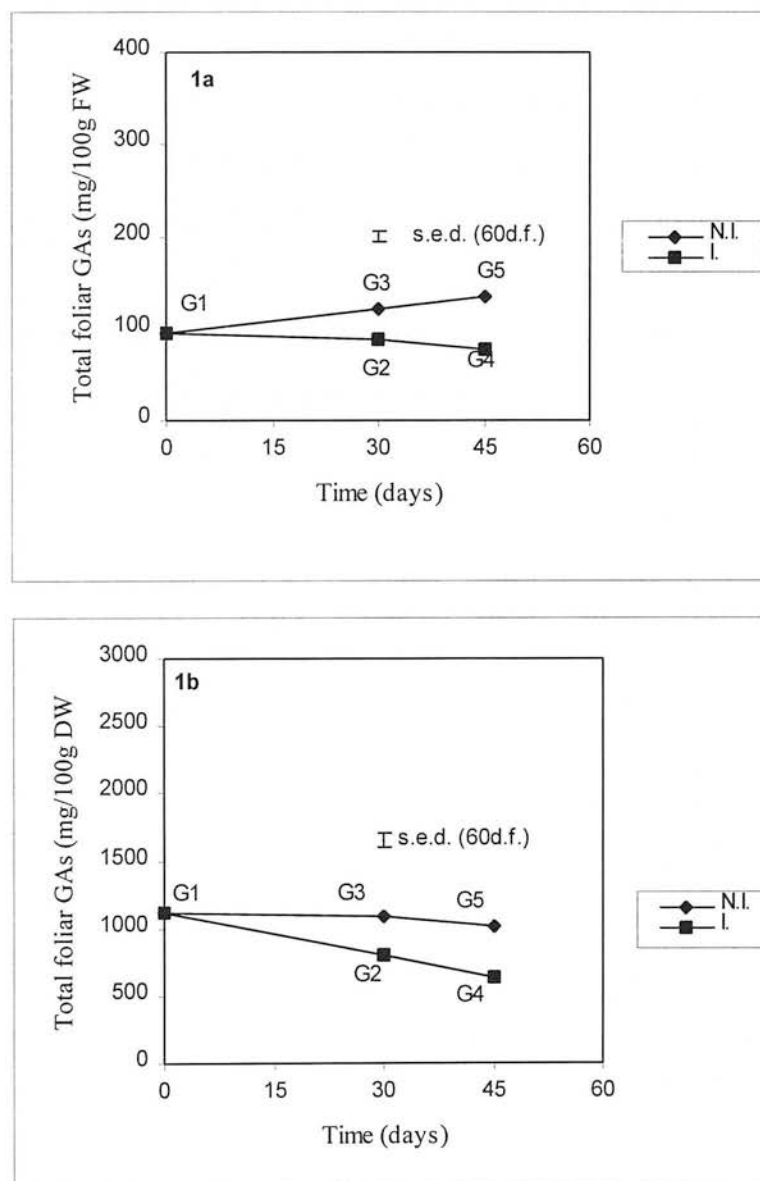
N₁: 50 mg total nitrogen/l solution, N₂: 100 mg total nitrogen/l solution, N₃: 200 mg total nitrogen/l solution, N₄: 300 mg total nitrogen/l solution

FW: fresh weight, DW: dry weight

The difference in green matter produced between plants grown in the N₄ treatment of G2 and G3 groups was also significant (P<0.01). These differences remained significant for plants grown in N₃ (P<0.01) and N₄ (P<0.001) nutrient solutions and harvested in G4 and G5 groups. Although the green matter produced by aphid-infested plants was decreased compared to that produced by non-infested plants grown in N₁ and N₂ nitrogen treatments, this decrease was found not to be significant (P>0.05) in this experiment. The significant (P<0.01) difference observed in the dry matter produced between the aphid-infested plants (G2 & G4) (Table 4.1b) was mainly due to the significant (P<0.01) difference observed in N₃ and N₄ treatments. No significant (P>0.05) differences between the aphid-infested plants were observed in N₁ and N₂ treatments. The highly significant (P<0.001) difference observed between the non-infested plants (G3 & G5) was due to the highly significant (P<0.001) differences observed in N₂ and N₄ treatments.

4.3.2 Effects of nitrogen fertilisation and aphid infestation on total glycoalkaloid production

In general, aphid infestation induced an overall reduction of the total foliar glycoalkaloid production of potato plants as this was expressed in mg of glycoalkaloids produced / 100g fresh (FW) or dry (DW) weight. Figure 4.1a shows the effects of aphid infestation on the total foliar glycoalkaloid production across all nitrogen levels applied.



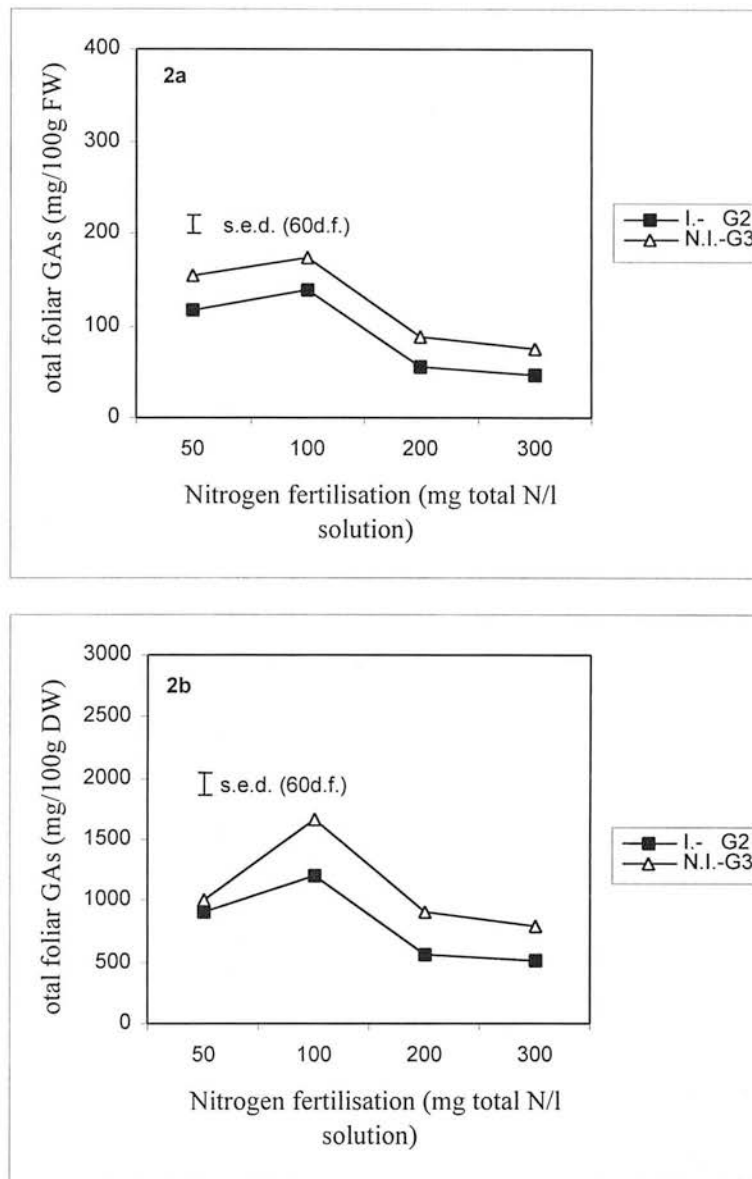
Figures 4.1a & 4.1b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids (GAs) produced by aphid-infested (I.-G2 & G4) and non-infested (N.I.-G1, G3 & G5) plants harvested at three different time intervals.

Plants infested with aphids and harvested 30 days after the infestation (G2 treatment) produced significantly ($P<0.01$) decreased amounts of foliar glycoalkaloids compared to non-infested plants (G3 treatment) harvested at the same time (effect of aphid infestation-Figure 4.1a). The decrease in the glycoalkaloid production was highly significant ($P<0.001$) between G4 (aphid-infested) and G5 (non-infested) plants harvested 45 days after the aphid infestation (after-effect of aphid infestation).

Similar reductions of glycoalkaloid production were observed when this was expressed as mg of glycoalkaloids produced per 100g dry weight (Figure 4.1b). The difference between the glycoalkaloids produced in G2 (infested) and G3 (non-infested) plants, as well as that between G4 (infested) and G5 (non-infested) plants were significant ($P<0.01$) and highly significant ($P<0.001$) respectively.

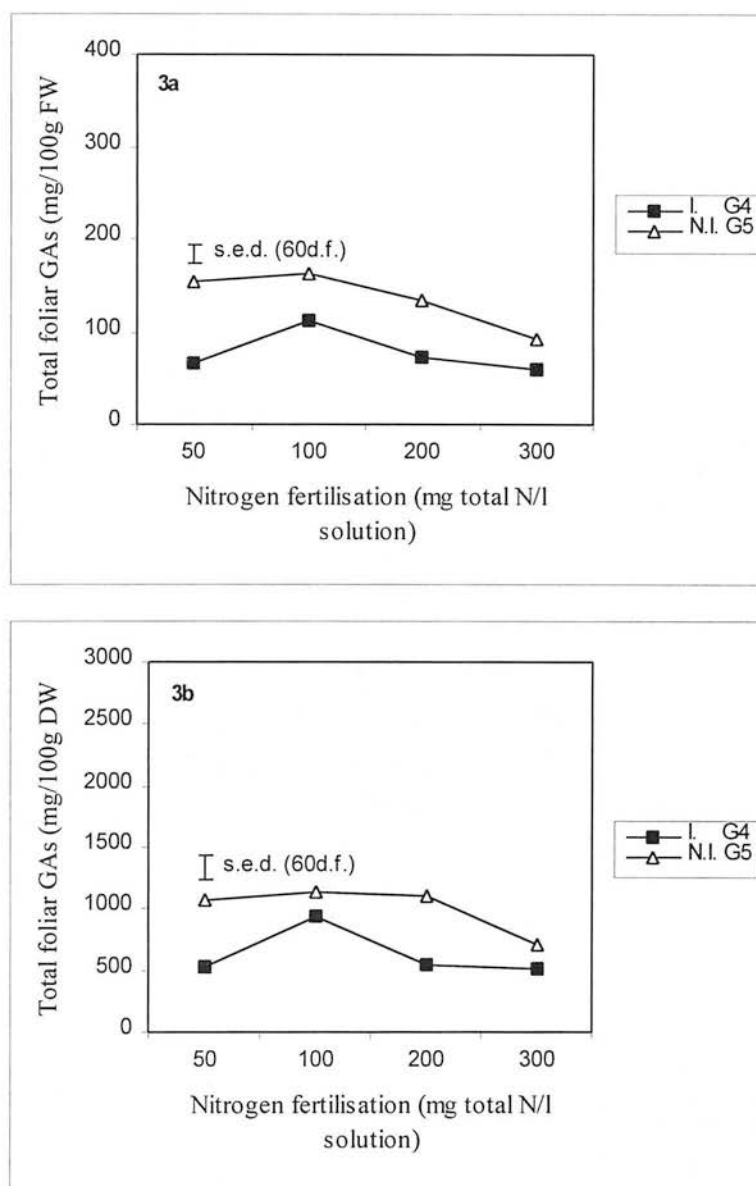
However when the effect of aphid infestation on the total FW foliar glycoalkaloid production was examined separately for every nitrogen treatment, no significant differences ($P>0.05$) were observed between aphid-infested (G2) and non-infested (G3) plants for any of the nitrogen fertilisation levels applied (Figure 4.2a). The highest amount of glycoalkaloids was produced in N_2 treatment though the difference between N_1 and N_2 treatments was not significant ($P>0.05$) for both aphid-infested and non-infested plants.

The differences between N_1 to N_3 and N_4 treatments were significant ($P<0.01$) and highly significant ($P<0.001$) respectively. In addition the differences between N_2 to N_3 and N_4 treatments were highly significant ($P<0.001$) for both aphid-infested (G2) and non-infested (G3) plants. By contrast, a significant difference ($P<0.05$) was observed between aphid-infested and non-infested plants on N_2 treatment when the glycoalkaloid production was calculated as mg produced per 100g dry weight (DW) (Figure 4.2b).



Figures 4.2a & 4.2b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids (GAs) produced by aphid-infested (G2) and non-infested (G3) plants for four nitrogen (N_1 , N_2 , N_3 and N_4) fertilisation levels (Exp. 1).

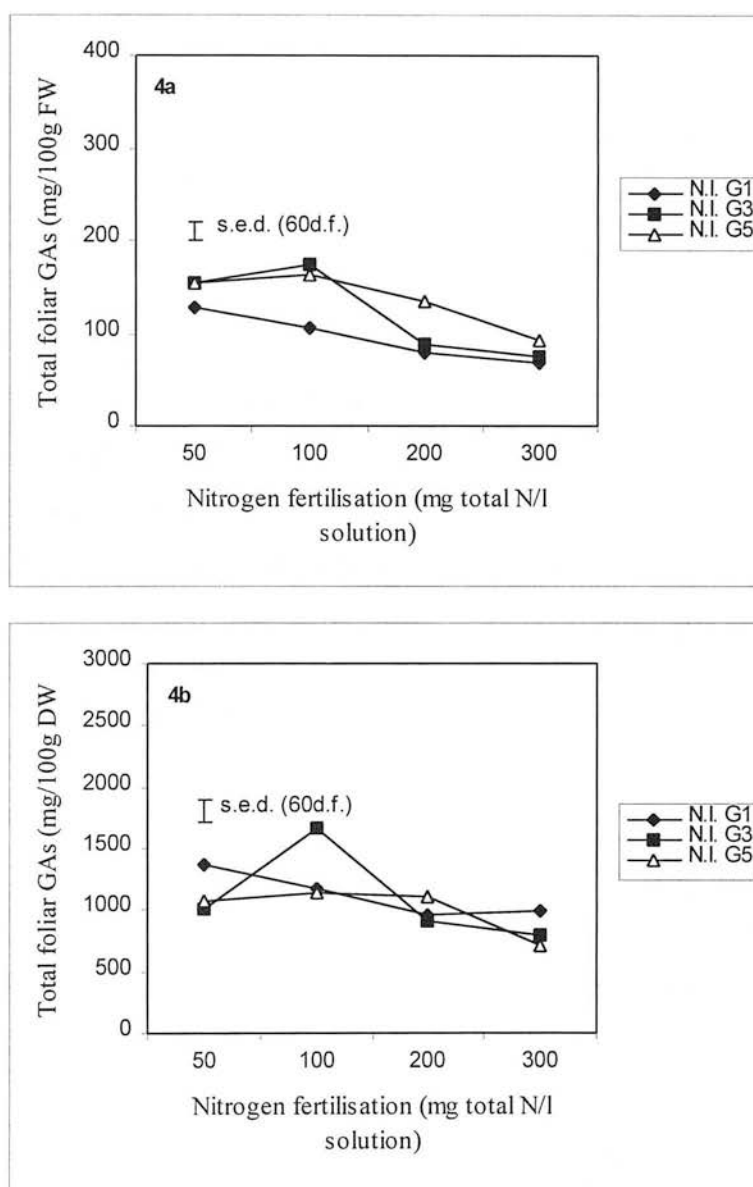
The after effects of aphid infestation are presented in Figures 4.3a and 4.3b. The differences in the glycoalkaloid production between aphid-infested (G4) and non-infested (G5) plants varied across nitrogen treatments. Aphid-infested plants produced lesser amounts of glycoalkaloids than those produced by non-infested plants. In particular when glycoalkaloid production was expressed on a fresh weight basis (Figure 4.3a), the differences were highly significant in the lowest N_1 ($P < 0.001$) treatment and significant in the medium N_3 ($P < 0.01$) and low N_2 ($P < 0.05$) treatments.



Figures 4.3a & 4.3b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids (GAs) produced by aphid-infested (G4) and non-infested (G5) plants for four nitrogen (N_1 , N_2 , N_3 and N_4) fertilisation levels (Exp. 1).

No significant differences ($P > 0.05$) were observed in the highest N_4 nitrogen treatment. Similar results were achieved when the glycoalkaloid production was expressed as mg produced per 100 g dry weight (Figure 4.3b). Significant differences ($P < 0.01$) between aphid-infested (G4) and non-infested (G5) plants were observed in N_1 and N_3 treatments while no significant differences ($P > 0.05$) were observed in N_2 and N_4 treatments. The amounts of total glycoalkaloids produced increased with time or developmental stage of non-infested plants (Figure 4.1a). In

particular, non-infested plants which approached the onset of flowering (G3 treatment) produced significantly ($P<0.01$) more glycoalkaloids compared to those produced by plants harvested at an earlier developmental stage (full development of the second true leaf-G1 treatment).



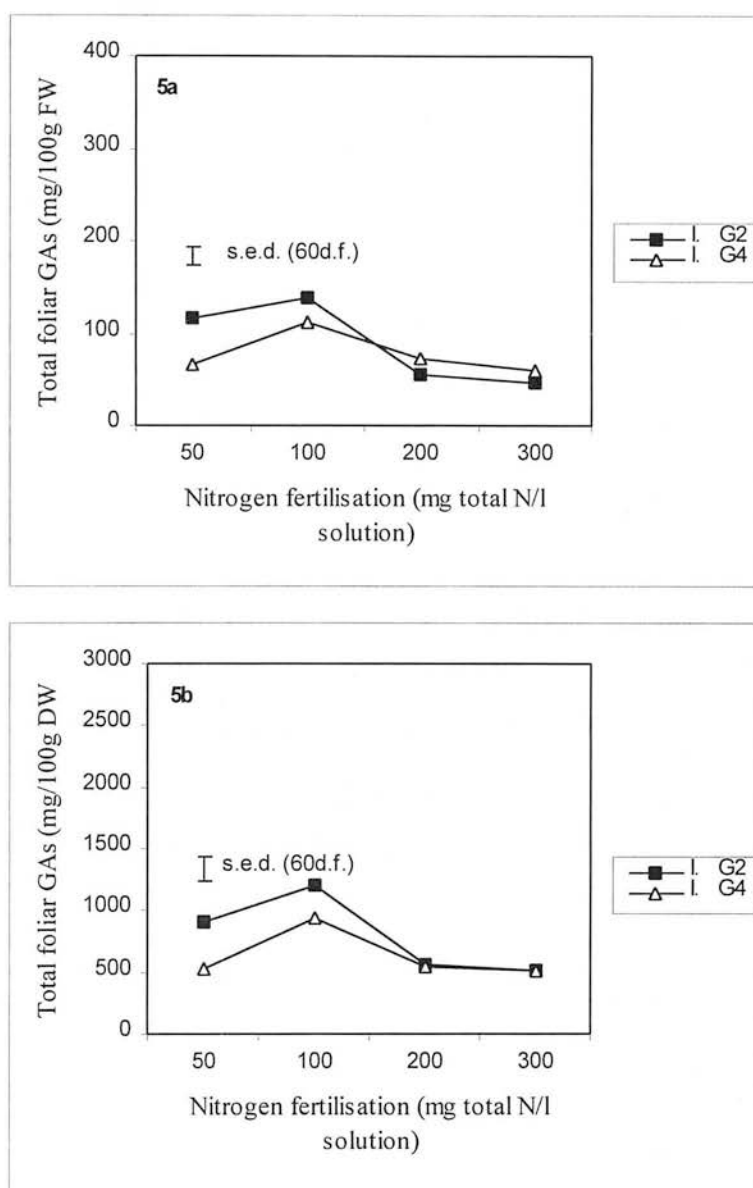
Figures 4.4a & 4.4b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids (GAs) produced by non-infested plants grown in four different nutrient solutions (N_1 , N_2 , N_3 and N_4) and harvested at three different developmental stages (G1, G3 and G5) (Exp. 1).

Furthermore a highly significant ($P<0.001$) difference was observed between G1 and G5 treatments. No significant ($P>0.05$) difference was observed between G3 and G5 treatments. By contrast to the results obtained when the glycoalkaloids were

expressed on a fresh weight basis, no significant ($P>0.05$) differences were observed between G1 and G3 to G5 plants when the glycoalkaloid production was measured as mg/100g DW (Figure 4.1b). The effect of developmental stage on the total foliar glycoalkaloid production was examined separately for every nitrogen treatment. No significant differences ($P>0.05$) were observed in the FW foliar glycoalkaloids produced in the lowest (N_1) and highest (N_4) treatments between G1, G3 and G5 plants (Figure 4.4a). However in the lowest nitrogen (N_1) treatment, G3 and G5 plants produced more –although not significant ($P>0.05$)- glycoalkaloids to those produced by G1.

Plants grown in the low (N_2) nitrogen treatment produced significantly more glycoalkaloids in G3 ($P<0.001$) and G5 ($P<0.01$) treatments compared to that produced in G1 treatment. No significant ($P>0.05$) differences were observed in the glycoalkaloids produced between G3 and G5 treatments. In addition non-infested plants grown in the medium (N_3) nitrogen treatment produced slightly different results from those obtained on N_2 treatment. G1 and G3 plants produced approximately the same amounts of glycoalkaloids, while G5 plants produced significantly more glycoalkaloids to that produced by G1 ($P<0.01$) and G3 ($P<0.05$) plants. However no significant ($P>0.05$) differences were observed when the glycoalkaloid production was expressed as mg produced per 100g dry weight for any of the nitrogen treatments, with an exception observed in N_2 , where G3 plants produced significantly more ($P<0.05$) glycoalkaloids to those produced by G1 and G5 plants (Figure 4.4b).

Aphid infestation induced a slight temporary increase of the glycoalkaloids produced in aphid-infested plants. Plants grown in G2 treatment produced more glycoalkaloids than plants grown in G4 treatment (Figures 4.1a & 4.1b). However this increase was not significant ($P>0.05$) when the glycoalkaloid production was expressed in fresh (Figure 4.1a) or dry (Figure 4.1b) weight basis. The fresh and dry weight glycoalkaloid values of G2 and G4 treatments were plotted against nitrogen fertilisation levels in figures 4.5a and 4.5b.



Figures 4.5a & 4.5b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids (GAs) produced by aphid-infested plants grown in four different nutrient solutions (N_1 , N_2 , N_3 and N_4) and harvested at two different developmental stages (G2 and G4) (Exp. 1).

Plants grown in the lowest (N_1) nitrogen nutrient solution and harvested 30 days after the aphid infestation (G2) produced significantly ($P < 0.01$) increased amounts of glycoalkaloids compared to that produced by plants grown in the same nutrient solution but harvested 45 days after aphid infestation (G4). In the low (N_2) nitrogen fertilisation treatment, G2 plants produced more although not significant ($P > 0.05$) glycoalkaloids compared to those produced by G4 plants. This situation was reversed in the medium (N_3) and high (N_4) nitrogen treatments where G2 plants

produced slightly less glycoalkaloids compared to those produced by G4 plants. Similar results were produced when the glycoalkaloid production was expressed on a dry weight basis, where the difference between G2 and G4 plants was significant ($P<0.05$) in the N_1 treatment (Figure 4.5b). No significant ($P>0.05$) differences were observed between G2 and G4 plants in the N_2 treatment while almost the same amounts -indicated by overlapping-of glycoalkaloid values were produced in the N_3 and N_4 treatments.

4.3.3 Effects of nitrogen fertilisation and aphid infestation on individual glycoalkaloid production

Table 4.2a shows the effects of nitrogen fertilisation on the individual foliar glycoalkaloids produced by the potato plants expressed as mg produced per 100g fresh weight. The total amounts of α -solanine and α -chaconine decreased significantly ($P<0.001$) with increasing nitrogen fertilisation. However the level of nitrogen in the nutrient solution did not affect the α -solanine to α -chaconine ratio of potato plants.

Table 4.2a. Effects of nitrogen (N) fertilisation on individual foliar glycoalkaloids (GAs) produced by potato plants cv. King Edward

Nutrient solutions	α -solanine mg/100g FW	α -chaconine mg/100g FW	S:C ratio
N_1	41.2	82.3	33:67
N_2	46.2	92.8	33:67
N_3	28.4	56.7	33:67
N_4	22.5	45.7	33:67
SED	2.95	5.8	0.007
DF	60	60	60
P	***	***	NS

SED: standard error of difference between means, DF: degrees of freedom
 *** $P<0.001$, NS: Non significant
 N_1 : 50 mg total nitrogen/l solution, N_2 : 100 mg total nitrogen/l solution, N_3 : 200 mg total nitrogen/l solution, N_4 : 300 mg total nitrogen/l solution
 FW: Fresh weight

Aphid infestation did not have any significant effect ($P>0.05$) on the proportion of α -solanine to α -chaconine (Table 4.2b). The same result was obtained between aphid-infested and non-infested plants harvested at different times (G2 to G3 and G4 to G5 respectively). However the harvesting time altered the α -solanine to α -chaconine

ratio. In particular plants harvested in G1 group produced significantly less amounts of α -solanine compared to the amounts produced by plants harvested after 30 ($P<0.01$) or 45 ($P<0.001$) days (G3 and G5 respectively). In addition the difference in the amounts of α -solanine produced between G2 to G4 and G3 to G5 plants was significant ($P<0.01$). No significant ($P>0.05$) effect of the interaction of nitrogen fertilisation x aphid infestation on the proportion of α -solanine to α -chaconine was observed (Table 4.2c).

Table 4.2b. Effects of aphid infestation on individual foliar glycoalkaloids (GAs) produced by five different groups of aphid infested (G2, G4) and non-infested (G1, G3, G5) potato plants cv. King Edward

G1			G2			G3			G4			G5		
α -sol	α -cha	S:C	α -sol	α -cha	S:C	α -sol	α -cha	S:C	α -sol	α -cha	S:C	α -sol	α -cha	S:C

30.7	64.7	32:68	29.3	60	33:67	40.7	81.7	33:67	25.8	51.2	34:66	46.5	89.1	34:66
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SED

DF

P

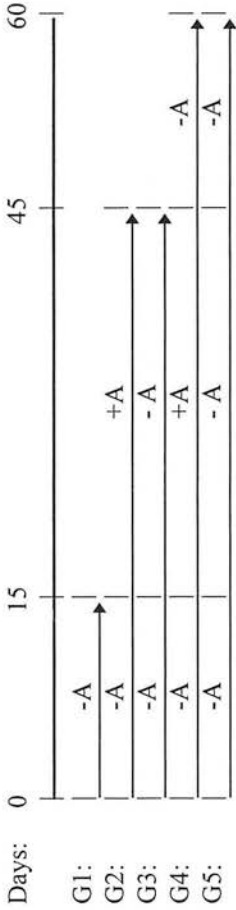
α -solanine: 3.3, α -chaconine:6.5, S:C: 0.008

60

α -solanine: ***, α -chaconine: ***, S:C ratio:***

SED: standard error of difference between means, DF: degrees of freedom

*** P<0.001,



α -sol: α -solanine (mg/100g FW), α -cha: α -chaconine (mg/100g FW), S:C: ratio of α -solanine to α -chaconine FW: Fresh weight

Table 4.2c. Interaction effects of nitrogen (N) fertilisation x aphid infestation on individual foliar glycoalkaloids (GAs) produced by five different groups of aphid infested (G2, G4) and non-infested (G1, G3, G5) potato plants cv. King Edward

Nutrient solutions	G1				G2				G3				G4				G5			
	α -sol	α -cha	S:C		α -sol	α -cha	S:C		α -sol	α -cha	S:C		α -sol	α -cha	S:C		α -sol	α -cha	S:C	
N ₁	41.3	87.0	32:68		38.2	78.9	33:67		51.4	102.0	34:66		21.9	43.1	34:66		53.3	100.3	35:65	
N ₂	33.6	72.4	32:68		46.2	92.6	33:67		57.9	116.5	34:66		37.2	75.4	33:67		56.3	107.0	35:65	
N ₃	26.0	52.4	33:67		17.3	36.9	32:68		29.2	58.7	33:67		24.2	47.6	34:67		45.4	87.9	34:66	
N ₄	21.9	46.9	32:68		15.5	31.5	33:67		24.1	49.7	33:67		20.1	38.9	34:67		31.1	61.3	34:66	
SED	α -solanine: 6.6, α -chaconine: 13, S:C ratio: 0.015																			
DF	60																			
P	α -solanine: NS, α -chaconine: NS, S:C ratio: NS																			
SED: standard error of difference between means, DF: degrees of freedom																				
NS: Non significant																				
Days:	0	15			45				60											
G1:		-A																		
G2:		-A																		
G3:		-A																		
G4:		-A																		
G5:		-A																		

N₁: 50 mg total nitrogen/l solution, N₂: 100 mg total nitrogen/l solution, N₃: 200 mg total nitrogen/l solution, N₄: 300 mg total nitrogen/l solution
 α -sol: α -solanine (mg/100g FW). α -cha: α -chaconine (mg/100g FW). S:C: ratio of α -solanine to α -chaconine FW: Fresh weight

N₁:50 mg total nitrogen/l solution, N₂: 100 mg total nitrogen/l solution, N₃: 200 mg total nitrogen/l solution, N₄: 300 mg total nitrogen/l solution
 α -sol: α -solanine (mg/100g FW), α -cha: α -chaconine (mg/100g FW), S:C: ratio of α -solanine to α -chaconine FW: Fresh weight

4.3.4 Aphid performance

Figure 4.6 shows the adult cumulative fecundity of *M. persicae* on potato plants cv. King Edward grown in four different nitrogen solutions (N_1 , N_2 , N_3 and N_4). During the first six days of reproduction no significant ($P>0.05$) differences were observed on the aphid performance for any of the four nitrogen treatments applied.

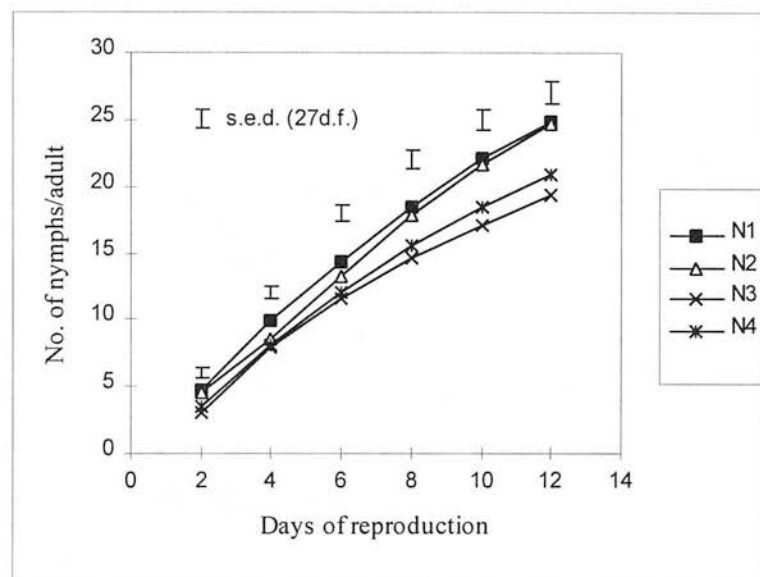


Figure 4.6. Mean cumulative fecundity of *Myzus persicae* adults infesting potato plants grown in four different nitrogen solutions.

A significant ($P<0.05$) effect on aphid performance was observed after eight days of reproduction. This was mainly due to the significantly elevated numbers of nymphs produced on plants grown in N_1 treatment compared to those produced by aphids which infested plants grown in N_3 and N_4 treatments. In addition a significant ($P<0.05$) difference was observed between the numbers of offspring produced in N_2 and N_3 treatments. In total the aphids which infested the plants grown in the low nitrogen solutions (N_1 and N_2) produced significantly more offspring compared to that produced by aphids which infested plants grown in N_3 ($P<0.01$) and N_4 ($P<0.05$) solutions (Figure 4.6 and Table 4.3). However no significant ($P>0.05$) differences were observed on the intrinsic rate of natural increase and on the mean daily number of offspring which was produced by aphids which infested plants grown in the different nitrogen treatments.

Table 4.3. Fecundity of *Myzus persicae* adults fed on potato plants cv. King Edward grown in four different nutrient solutions

Nutrient solutions	Mean daily offspring (nymphs/adult)	Mean total progeny (nymphs/adult)	Intrinsic rate of natural increase (r_m)
N ₁	2.07	24.75	0.25
N ₂	1.80	24.71	0.24
N ₃	1.62	19.37	0.23
N ₄	1.76	20.87	0.22
SED	0.22	1.64	0.02
DF	28	28	28
P	NS	**	NS

SED: standard error of difference between means, DF: degrees of freedom
N₁:50 mg total nitrogen/l solution, N₂: 100 mg total nitrogen/l solution, N₃: 200 mg total nitrogen/l solution, N₄: 300 mg total nitrogen/l solution
** P<0.01, NS Non significant

Experiment 2

4.3.5 Effects of nitrogen fertilisation, aphid infestation and cultivar on green and dry matter

Table 4.4a shows the effects of nitrogen fertilisation and aphid infestation on the green and dry matter produced by potato plants. Elevated amounts of nitrogen increased significantly ($P<0.01$) the overall green matter produced by both potato cultivars. In addition increasing nitrogen fertilisation reduced the amounts of dry matter produced by both potato cultivars. However this reduction was not significant ($P>0.05$).

Table 4.4a. Interaction effects of nitrogen (N) fertilisation x cultivar on the green (FW) and dry matter (DW/FW) produced by potato plants cvs. King Edward (KE) and Maris Piper (MP)

Cultivar	Nutrient solutions	Green matter FW (g)	Dry matter (DW/FW)
KE	N ₂	27.74	0.121
KE	N ₄	60.28	0.108
MP	N ₂	25.23	0.110
MP	N ₄	68.70	0.102
SED		2.5	0.005
DF		60	60
P		**	NS

SED: standard error of difference between means, DF: degrees of freedom

** P<0.01, NS: Non significant

N₂: 100 mg total nitrogen/l solution, N₄: 300 mg total nitrogen/l solution

FW: fresh weight DW: dry weight

Aphid infestation induced a reduction in the amounts of green matter produced (Table 4.4b). In the low nitrogen treatment (N_2), aphid-infested plants (G2) from both varieties (KE + MP) produced significantly ($P<0.05$) less amounts of green matter compared to those produced by non-infested plants (G3). This difference was highly significant ($P<0.001$) between G4 and G5 treatments. In the high nitrogen N_4 treatment the difference between G2 and G3 treatments and that between G4 and G5 treatments were highly significant ($P<0.001$) as well. Aphid infestation induced an increase in the dry matter produced by aphid-infested plants (Table 4.4b). In the low nitrogen treatment (N_2), aphid-infested plants (G2 & G4) from both varieties (KE + MP) produced significantly ($P<0.05$) elevated amounts of dry matter compared to that produced by non-infested plants (G3 & G5). In the high nitrogen treatment (N_4) the difference between G2 and G3 treatments and that between G4 and G5 treatments were highly significant ($P<0.001$).

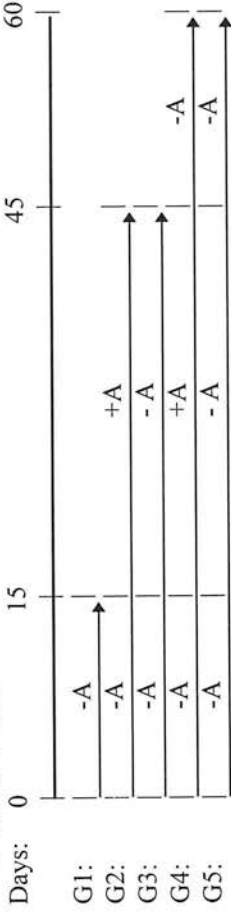
No significant differences ($P>0.05$) in the green matter produced were observed for both aphid-infested and non-infested plants between the two potato cultivars (Table 4.4c). An exception was observed in the G4 treatment where cv. King Edward produced significantly ($P<0.001$) less amounts of green matter compared to those produced by cv. Maris Piper. This was mainly due to the significantly ($P<0.001$) decreased green matter produced in cv King Edward in the high nitrogen N_4 treatment (Table 4.4d). As for the green matter no significant differences ($P>0.05$) in the dry matter were observed for both aphid-infested and non-infested plants between the two potato cultivars (Table 4.4c). An exception was observed in the G4 treatment where cv. King Edward produced significantly ($P<0.001$) more dry matter compared to that produced by cv. Maris Piper. As in the previous experiment no significant ($P>0.05$) differences were observed in the green and dry matter produced between N_2 and N_4 treatments in the non-infested plants harvested in the G1 group for both potato cultivars (KE & MP) and both nitrogen levels applied (N_2 & N_4) (Table 4.4d). There was no significant ($P>0.05$) interaction between nitrogen fertilisation, aphid infestation and cultivar on the green and dry matter produced by both potato cultivars (Table 4.4d).

Table 4.4b. Interaction effects of nitrogen (N) fertilisation x aphid infestation on the green (FW) and dry matter (DW/FW) produced by aphid infested (G2, G4) and non-infested (G1, G3, G5) potato plants cvs. King Edward (KE) and Maris Piper (MP)

Cultivars	Nutrient solutions	Green matter FW (g)					Dry matter (DW/FW)				
		G1	G2	G3	G4	G5	G1	G2	G3	G4	G5
KE+MP	N ₂	8.38	27.07	35.90	20.58	40.5	0.076	0.124	0.104	0.147	0.127
	N ₄	12.90	58.46	83.92	52.41	114.8	0.070	0.113	0.085	0.157	0.101
SED				3.95					0.008		
DF				60					60		
P				***					*		

SED: standard error of difference between means, DF: degrees of freedom

*** P<0.001, * P<0.05



N₂: 100 mg total nitrogen/l solution, N₄: 300 mg total nitrogen/l solution

FW: fresh weight DW: dry weight

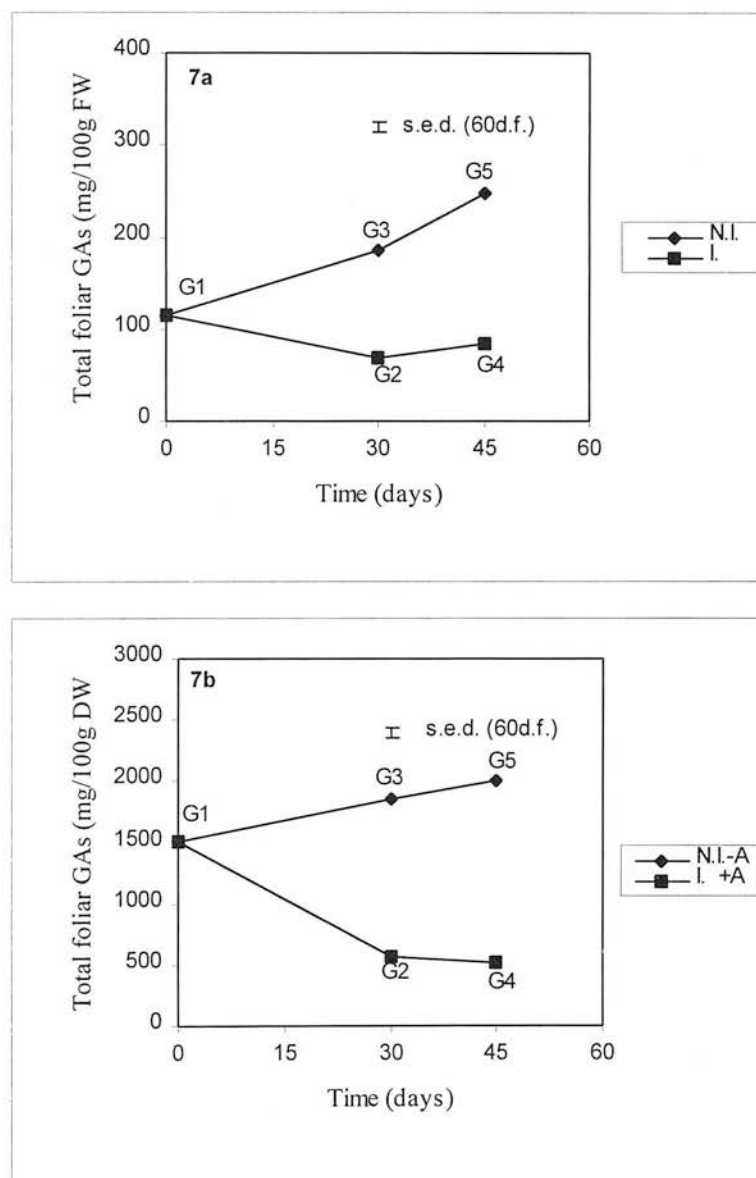
Table 4.4c. Interaction effects of cultivar x aphid infestation on the green (FW) and dry matter (DW/FW) produced by aphid infested (G2, G4) and non-infested (G1, G3, G5) potato plants cvs. King Edward (KE) and Maris Piper (MP)

Cultivar	Nutrient solutions	Green matter FW (g)					Dry matter (DW/FW)				
		G1	G2	G3	G4	G5	G1	G2	G3	G4	G5
KE	N ₂ + N ₄	10.90	40.78	61.07	28.09	79.21	0.075	0.118	0.096	0.169	0.116
MP	N ₂ + N ₄	10.36	44.74	58.74	44.90	76.05	0.072	0.119	0.093	0.135	0.113
SED				3.95					0.008		
DF				60					60		
P				**					*		
SED: standard error of difference between means, DF: degrees of freedom											
** P<0.01, * P<0.05,											
Days:		0	15			45			60		
G1:			-A								
G2:			-A		→	+A					
G3:			-A			-A					
G4:			-A			+A				-A	
G5:			-A			-A				-A	
N ₂ : 100 mg total nitrogen/l solution, N ₄ : 300 mg total nitrogen/l solution											
FW: fresh weight DW: dry weight											

4.3.6. Effects of nitrogen fertilisation and aphid infestation on total glycoalkaloid production

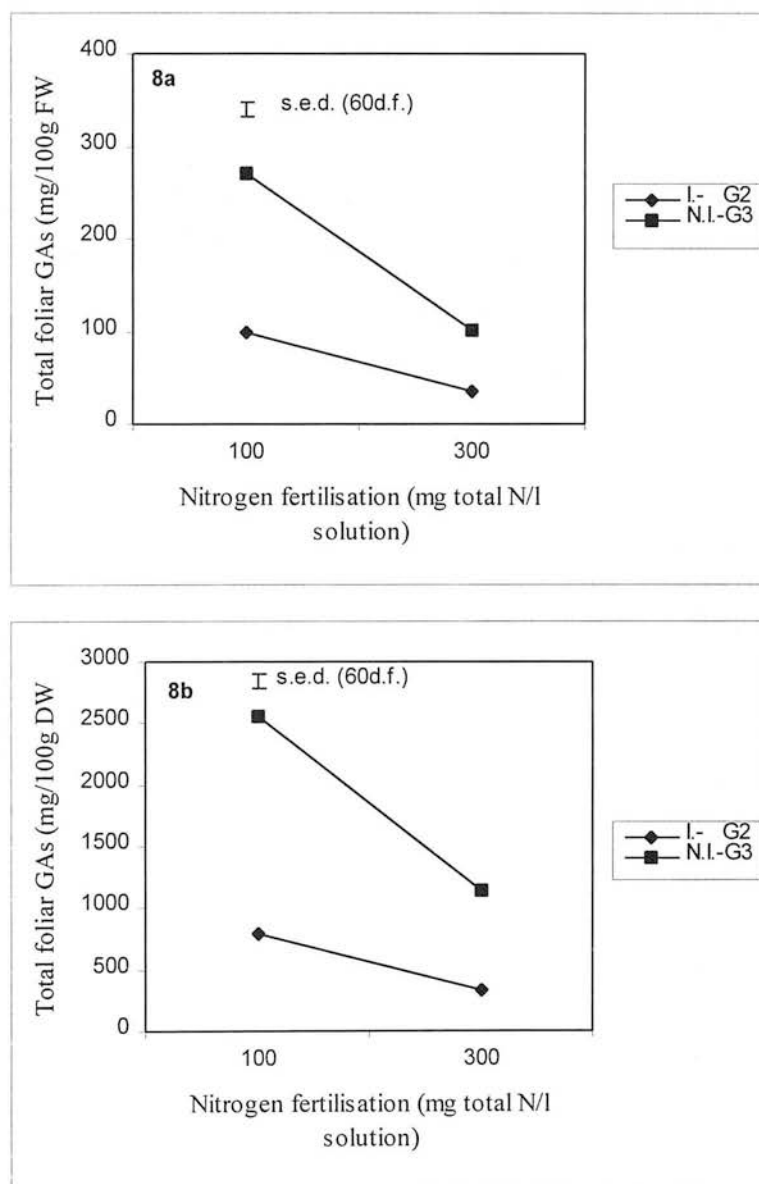
Potato cv. King Edward

As in the previous experiment aphid infestation induced an overall highly significant ($P < 0.001$) reduction of the total foliar fresh or dry weight glycoalkaloid production for potato cv. King Edward. Figures 4.7a and 4.7b show the effects of aphid infestation on the total foliar fresh and dry weight glycoalkaloid production of potato cv. King Edward for both nitrogen (N_2 and N_4) levels applied.



Figures 4.7a & 4.7b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids (GAs) produced by aphid-infested (I. - G2 & G4) and non-infested (N.I. - G1, G3 & G5) potato plants cv. King Edward harvested at three different time intervals.

The difference between aphid-infested (G2 and G4 treatments) to non-infested (G3 and G5 treatments) plants was highly significant ($P < 0.001$) when glycoalkaloid production was expressed in fresh or dry weight basis. In particular, plants harvested 30 days after the aphid infestation (G2 treatment) produced significantly ($P < 0.001$) less glycoalkaloids compared to the glycoalkaloids produced by non-infested plants harvested at the same time (G3 treatment). In addition plants harvested 15 days later (after effect of aphid infestation - G4 treatment) still produced significantly ($P < 0.001$) less glycoalkaloids compared to non-infested plants (G5 treatment).



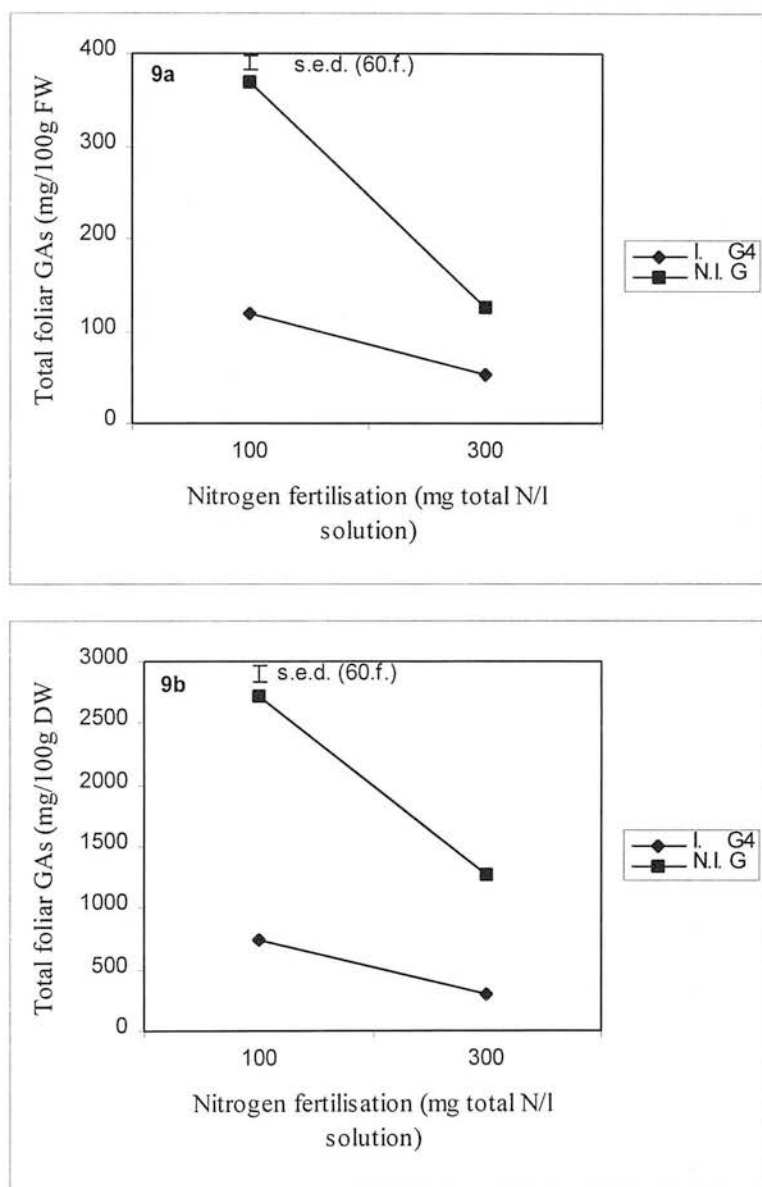
Figures 4.8a & 4.8b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids (GAs) produced by aphid-infested (G2) and non-infested (G3) potato plants cv. King Edward for two nitrogen (N_2 and N_4) fertilisation levels (Exp. 2).

By contrast to the results obtained from the previous experiment - where no significant differences were found between G2 and G3 plants for any of the nitrogen levels applied - the difference in the glycoalkaloid production between aphid-infested and non-infested plants was highly significant ($P<0.001$) when this was expressed separately for each of the two nitrogen levels applied (Figures 4.8a & 4.8b). In both nitrogen levels, aphid-infested plants G2 produced significantly ($P<0.001$) less glycoalkaloids compared to glycoalkaloids produced by non-infested plants G3. In addition both aphid-infested (G2) and non-infested (G3) plants grown in the low nitrogen treatment N_2 produced significantly ($P<0.001$) more glycoalkaloids compared to plants grown in the high nitrogen treatment N_4 .

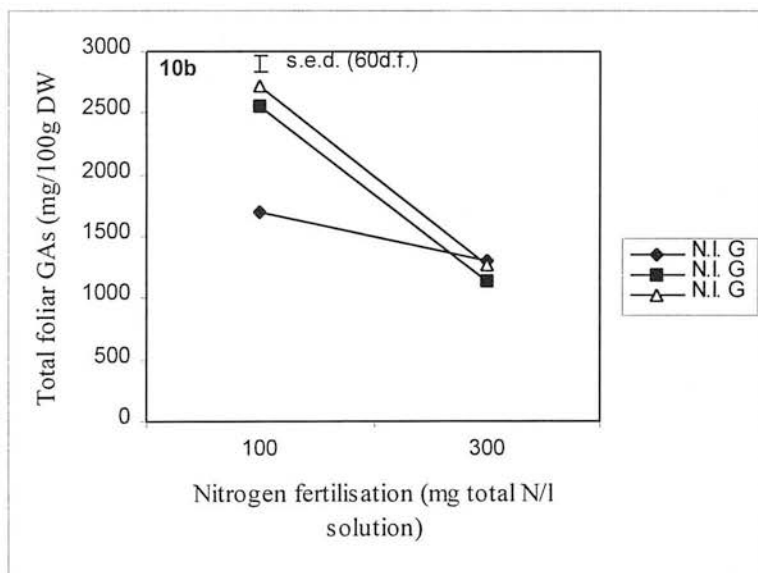
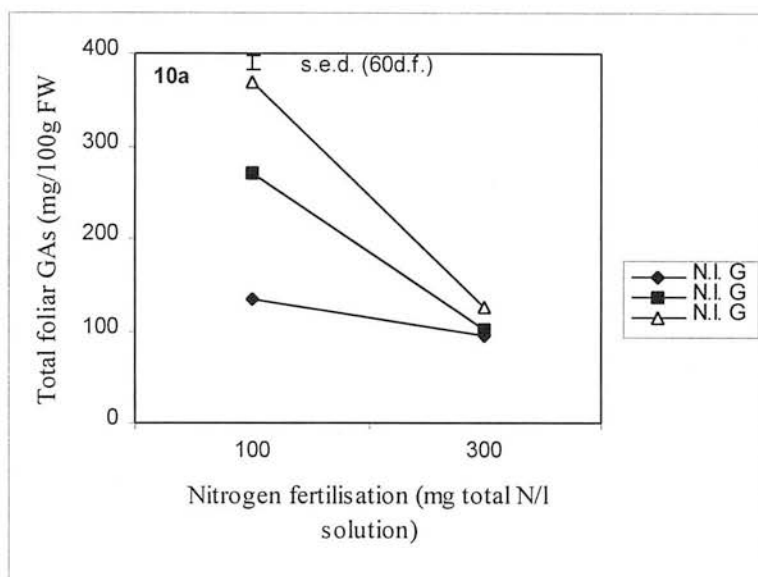
The same results were observed in plants harvested 15 days later (after effect of aphid infestation) where G4 treatment produced significantly ($P<0.001$) lower amounts of glycoalkaloids compared to those produced by G5 treatment for both nitrogen levels applied (Figures 4.9a & 4.9b). Time of harvesting induced a highly significant ($P<0.001$) increase of potato glycoalkaloids in the foliage of the non-infested plants grown in the low nitrogen treatment N_2 (Figure 4.10a). In particular G3 plants produced significantly ($P<0.001$) increased amounts of glycoalkaloids compared to those produced by G1 plants and significantly ($P<0.001$) reduced amounts of glycoalkaloids compared to those produced by G5 plants.

However no significant ($P>0.05$) differences were observed between G3 and G5 plants grown in the low nitrogen treatment N_2 while the glycoalkaloid production was expressed as mg produced per 100g dry weight (Figure 4.10b). The difference between G1 to G3 and G5 plants was highly significant ($P<0.001$). No significant ($P>0.05$) differences were observed between G1, G3 and G5 plants which were grown in the high nitrogen treatment N_4 . The results obtained from the previous exp. 1 showed that aphid infestation induced a slight temporary increase of the glycoalkaloids produced in infested plants of N_2 treatment (Figures 4.5a and 4.5b). However a slight although non-significant ($P>0.05$) decrease of the glycoalkaloids produced was observed in the G2 plants compared to those produced by G4 plants

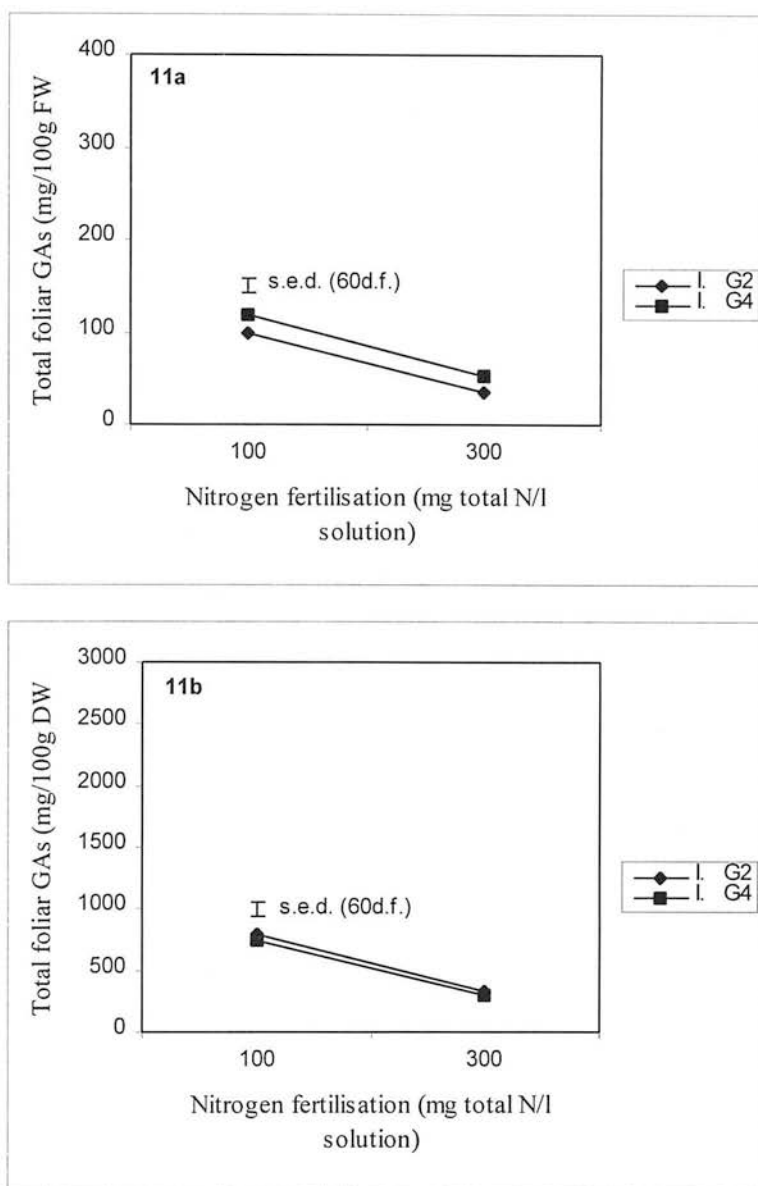
grown at the same nitrogen level (Figure 4.11a). By contrast when glycoalkaloid production was expressed as mg produced per 100g dry weight, G2 treatment produced a slight increase of the glycoalkaloid production compared to these produced by G4 treatment. However this increase was so imperceptible that both lines almost overlapped (Figure 4.11b).



Figures 4.9a & 4.9b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids (GAs) produced by aphid-infested (G4) and non-infested (G5) potato plants cv. King Edward for two nitrogen (N_2 and N_4) fertilisation levels (Exp. 2).



Figures 4.10a & 4.10b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids (GAs) produced by non-infested potato plants cv. King Edward, grown in two different nutrient solutions (N_2 and N_4) and harvested at three different developmental stages (G1, G3 and G5) (Exp. 2).

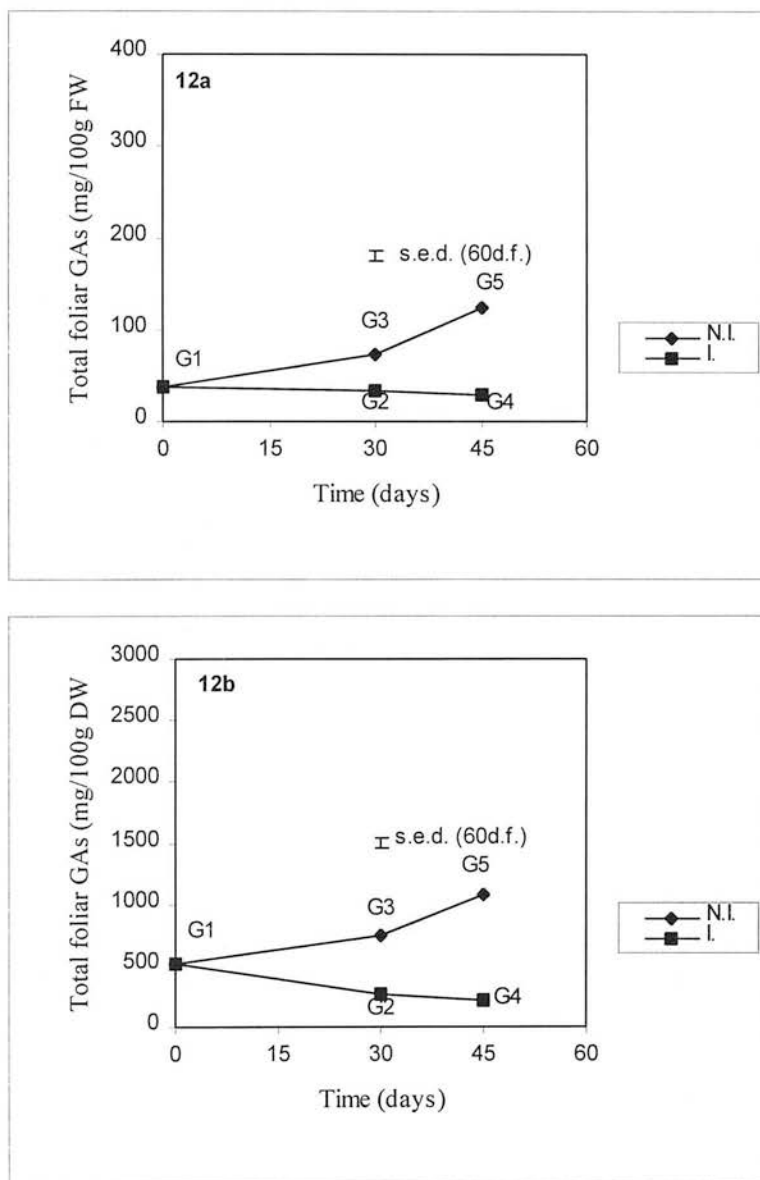


Figures 4.11a & 4.11b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids (GAs) produced by aphid-infested potato plants cv. King Edward, grown in two different nutrient solutions (N_2 and N_4) and harvested at two different developmental stages (G2 and G4) (Exp. 2).

Potato cv. Maris Piper

It had been expected from the second experiment of the previous chapter that potato cv. Maris Piper would produce significantly ($P < 0.001$) less amounts of glycoalkaloids to those produced by cv. King Edward for both nitrogen fertilisation levels applied. The results obtained from potato cv. Maris Piper were very similar to those obtained from cv. King Edward. Aphid-infested plants cv. Maris Piper

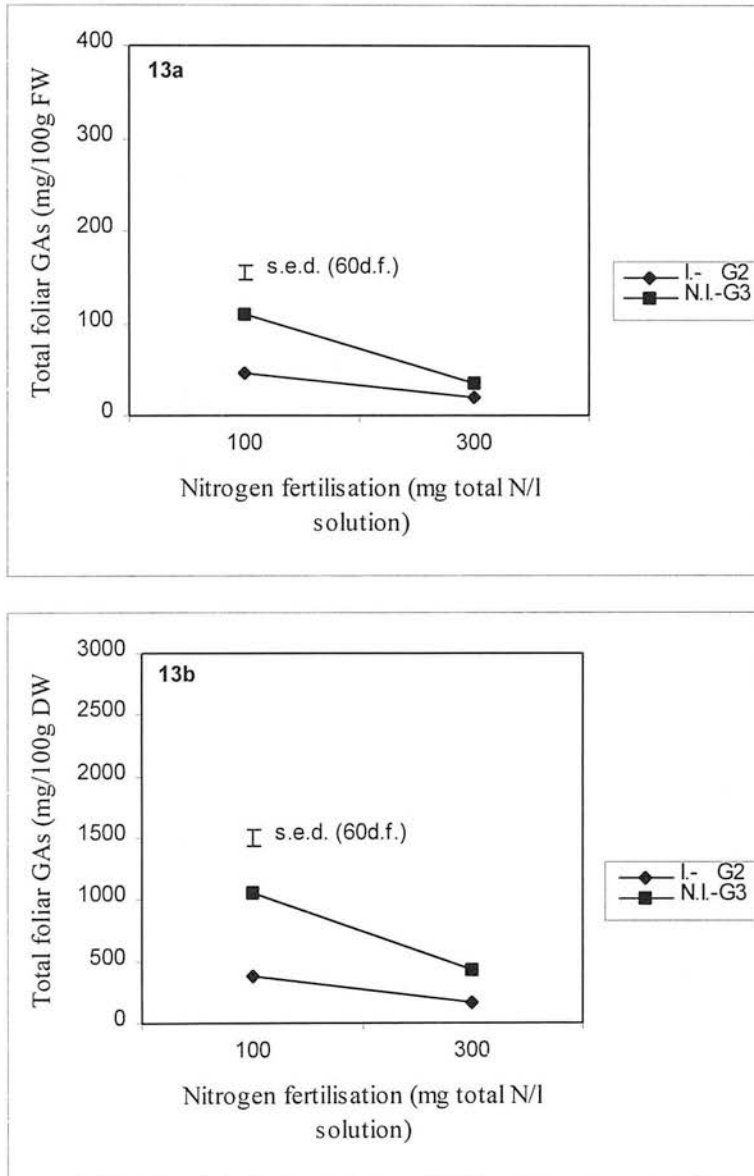
harvested 30 days after aphid infestation (G2) produced significantly ($P<0.001$) less fresh or dry weight glycoalkaloids compared to those produced by non-infested (G3) plants (Figures 4.12a & 4.12b).



Figures 4.12a & 4.12b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids (GAs) produced by aphid-infested (G2 & G4) and non-infested (G1, G3 & G5) potato plants cv. Maris Piper harvested at three different time intervals.

In addition a highly significant difference ($P<0.001$) was observed between G4 treatment and G5 treatment (after effect of aphid infestation). When the glycoalkaloid production of G2 and G3 treatments of cv. Maris Piper was plotted separately for each one of the nitrogen levels applied (Figures 4.13a & 4.13b), the

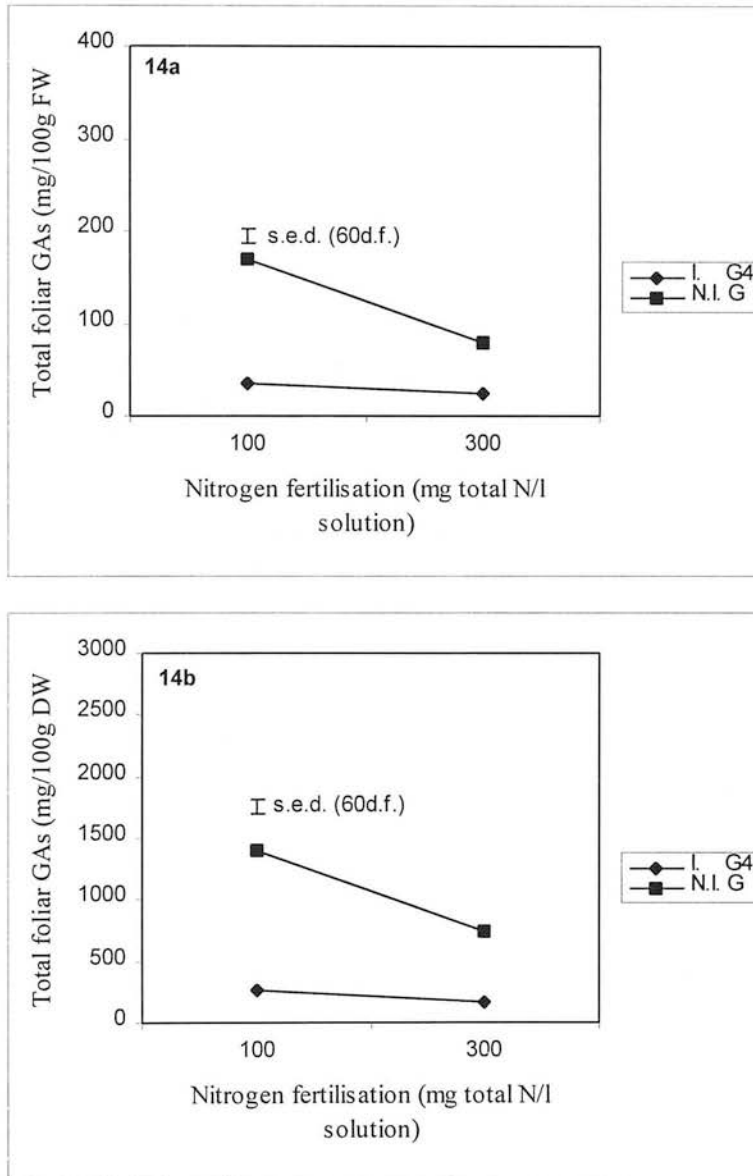
results obtained were similar to those obtained by cv. King Edward (Figures 4.8a & 4.8b). In particular a difference was observed between aphid-infested G2 and non-infested G3 plants grown in the low nitrogen treatment N_2 , where G2 plants produced significantly ($P<0.001$) less glycoalkaloids compared to these produced by G3 plants (Figure 4.13a).



Figures 4.13a & 4.13b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids (GAs) produced by aphid-infested (G2) and non-infested (G3) potato plants cv. Maris Piper for two nitrogen (N_2 and N_4) fertilisation levels (Exp. 2).

By contrast to potato cv. King Edward, no significant difference ($P>0.05$) was observed between G2 and G3 treatments grown in the high nitrogen treatment N_4 .

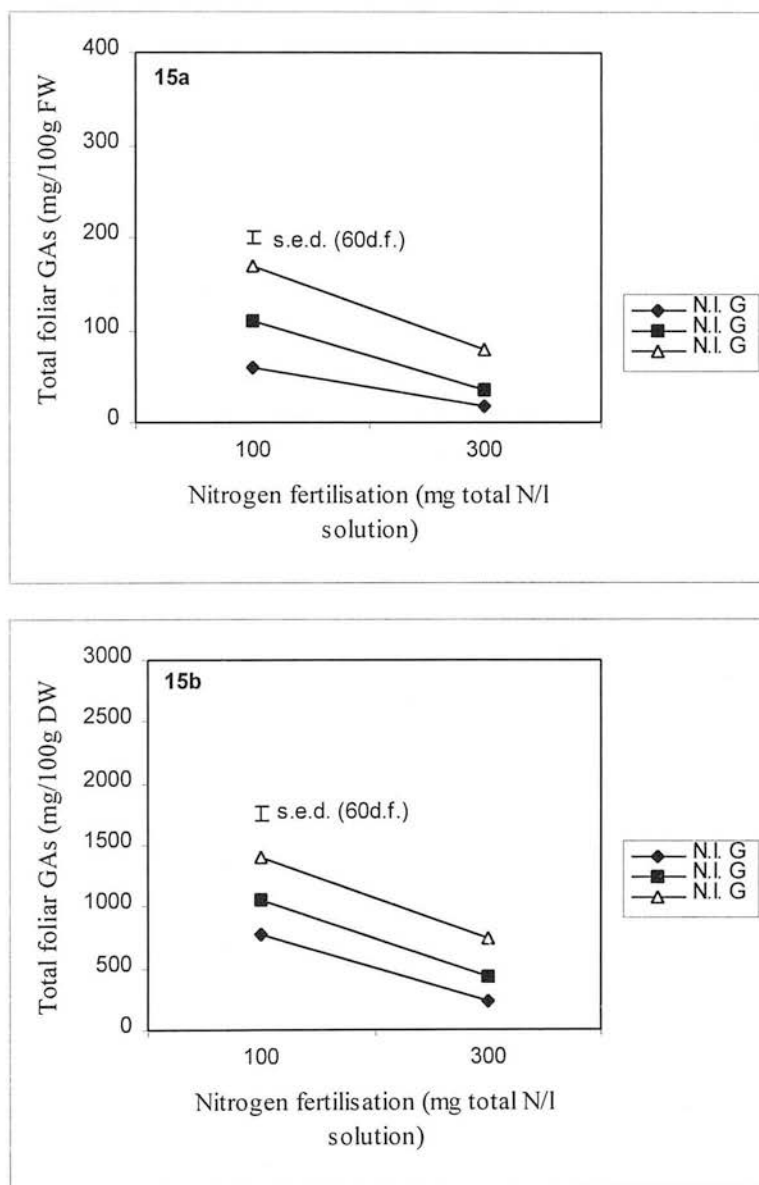
However aphid infested plants G2 produced slightly less glycoalkaloids to the amounts produced by the non infested G3 plants. This was reversed when the glycoalkaloid production was expressed in dry weight basis where a significant difference ($P<0.05$) was observed between G2 and G3 plants grown in the high nitrogen treatment N_4 (Figure 4.13b).



Figures 4.14a & 4.14b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids (GAs) produced by aphid-infested (G4) and non-infested (G5) potato plants cv. Maris Piper for two nitrogen (N_2 and N_4) fertilisation levels (Exp. 2).

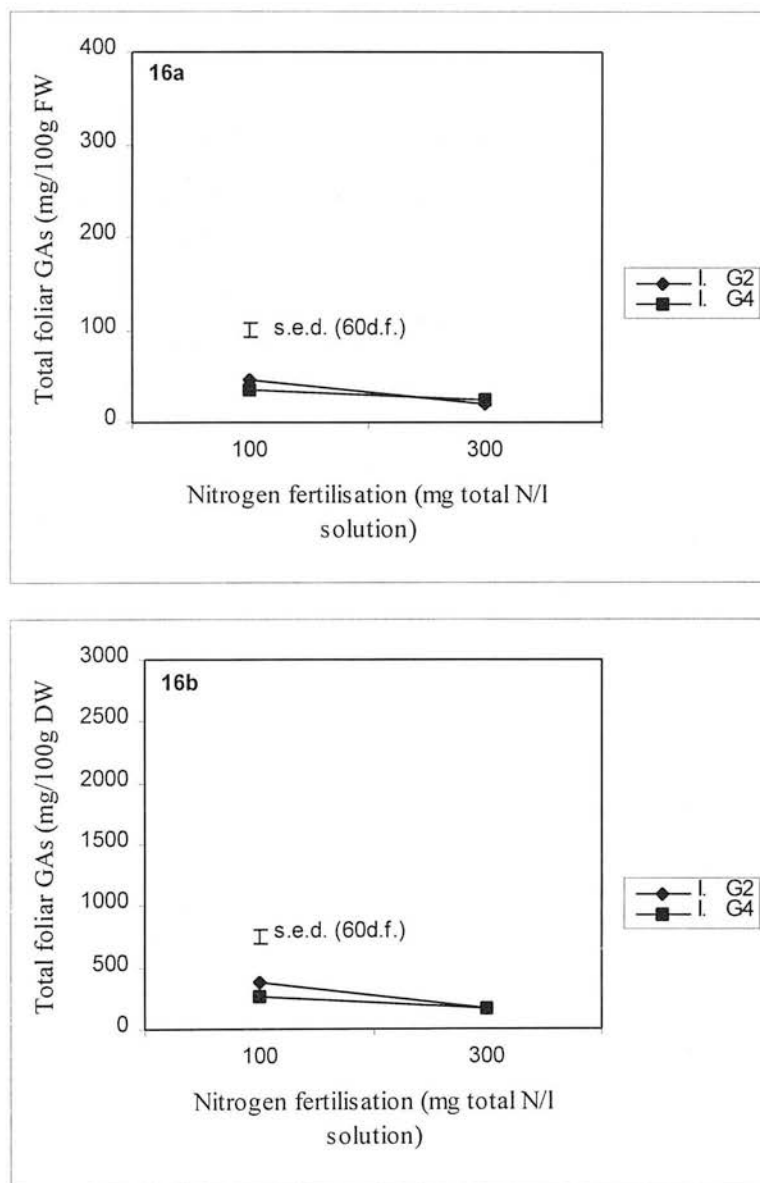
The same results were observed in plants harvested 15 days later (after effect of aphid infestation) where potato plants cv. Maris Piper of G4 treatment produced

significantly ($P < 0.001$) less amounts of glycoalkaloids compared to those produced by G5 treatment for both nitrogen levels (Figures 4.14a & 4.14b). Plants from the G1 treatment grown in the low nitrogen solution N_2 produced significantly ($P < 0.01$) less glycoalkaloids to those produced by G3 treatment. This difference became highly significant ($P < 0.001$) between G1 and G5 treatments as well as between G3 and G5 treatments (Figures 4.15a & 4.15b).



Figures 4.15a & 4.15b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids (GAs) produced by non-infested potato plants cv. Maris Piper, grown in two different nutrient solutions (N_2 and N_4) and harvested at three different developmental stages (G1, G3 and G5) (Exp. 2).

By contrast no significant difference ($P>0.05$) was observed between G1 and G3 treatments grown in the high nitrogen fertilisation N_4 while the difference between G1 and G5 treatments was highly significant ($P<0.001$) (Figures 4.15a & 4.15b). Aphid-infested plants cv. Maris Piper grown in the low N_2 treatment and harvested 30 days after the infestation (G2) produced slightly elevated although not significant ($P>0.05$) amounts of glycoalkaloids compared to those produced by G4 plants (Figures 4.16a & 4.16b).



Figures 4.16a & 4.16b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids (GAs) produced by aphid-infested potato plants cv. Maris Piper grown in two different nutrient solutions (N_2 & N_4) and harvested at two different developmental stages (G2 & G4) (Exp. 2).

This was observed as well when the glycoalkaloid production was expressed on a dry weight basis. Aphid-infested plants (G2 and G4) grown in the high nitrogen treatment N₄ harvested at different time points produced almost the same amounts of fresh or dry weight total foliar GAs.

4.3.7 Effects of nitrogen fertilisation, aphid infestation and cultivar on individual glycoalkaloid production

Table 4.5a shows the effects of nitrogen fertilisation on the individual foliar glycoalkaloids produced by both potato cultivars and expressed as mg produced per 100g fresh weight. As it was expected from the results obtained from the previous experiment (exp. 1) the total amounts of α -solanine and α -chaconine decreased significantly ($P < 0.001$) by increasing the nitrogen fertilisation applied in each potato cultivar.

Table 4.5a. Interaction effects of nitrogen (N) fertilisation x cultivar on individual foliar glycoalkaloids (GAs) produced by potato plants cvs. King Edward (KE) and Maris Piper (MP)

Cultivars	Nutrient solutions	solanine mg/100g FW	chaconine mg/100g FW	S:C ratio
KE	N ₂	67.5	130.7	34:66
KE	N ₄	28.1	53.1	35:65
MP	N ₂	34.6	49.1	41:59
MP	N ₄	14.2	20.6	41:59
SED		2.83	4.16	0.025
DF			60	
P		***	***	NS

SED: standard error of difference between means, DF: degrees of freedom

*** $P < 0.001$, NS: Non significant

N₂: 100 mg total nitrogen/l solution, N₄: 300 mg total nitrogen/l solution

sol: total ($\alpha + \beta$) solanine (mg/100g FW), cha: total ($\alpha + \beta$) chaconine (mg/100g FW), S:C: ratio of total solanine to total chaconine, FW: Fresh weight

FW: Fresh weight

In addition the level of nitrogen in the nutrient solution did not affect significantly ($P > 0.05$) the α -solanine to α -chaconine ratio for either of the two potato cultivars examined. However the proportion of α -solanine in the total glycoalkaloids produced by potato cv. Maris Piper was significantly ($P < 0.001$) higher to that produced by cv. King Edward for both nitrogen treatments. The two varieties

respond differently regarding how aphid infestation affected the α -solanine to α -chaconine proportion.

As in the previous experiment aphid infestation did not have any significant effect ($P>0.05$) on the proportion of α -solanine to α -chaconine produced by potato cv. King Edward (Table 4.5b). In particular potato plants harvested 30 days after the aphid infestation (G2) produced less -although not significantly ($P>0.05$)- amounts of α -solanine compared to the amounts produced by the non-infested plants (G3).

This was reversed in plants harvested 15 days later when G4 plants produced more -although not significant ($P>0.05$)- amounts of α -solanine compared to those produced by G5 treatment. By contrast, aphid-infested plants (G2 & G4) from potato cv. Maris Piper produced significantly more ($P<0.01$) amounts of α -solanine compared to those produced by non-infested plants (G3 & G5). Time of harvesting did not have any significant ($P>0.05$) effect on the proportion of α -solanine to α -chaconine for aphid-infested or non-infested plants. However G4 plants of potato cv. King Edward produced significantly ($P<0.05$) more α -solanine compared to that produced by G2 plants (Table 4.5b). No significant ($P>0.05$) effect of the interaction between aphid infestation, nitrogen fertilisation and cultivar was observed on the proportion of α -solanine to α -chaconine for either of the two potato cultivars examined (Table 4.5c).

Table 4.5b. Interaction effects of aphid infestation x cultivar on individual foliar glycoalkaloids (GAs) produced by five different groups of aphid infested (G2, G4) and non-infested (G1, G3, G5) potato plants cvs. King Edward (KE) and Maris Piper (MP)

Cultivars	G1		G2		G3		G4		G5		
	sol	cha	S:C	sol	cha	S:C	sol	cha	S:C	cha	S:C
KE	40.76	73.28	36:64	21.90	45.77	32:68	62.47	122.4	34:66	83.8	163.3
MP	16.02	21.59	41:59	14.06	18.18	44:56	28.60	43.7	40:60	50.4	74.1
SED	solanine: 4.47, chaconine: 6.57, S:C ratio: 0.04										
DF	60										
P	solanine: ***, chaconine: ***, S:C ratio: NS										
SED: standard error of difference between means, DF: degrees of freedom											
*** P<0.001, NS: Non significant											
Days:	0	15	45	60							
G1:		-A									
G2:		-A	→								
G3:		-A	→								
G4:		-A	→								
G5:		-A	→								
sol: total (α+β) solanine (mg/100g FW), cha: total (α+β) chaconine (mg/100g FW), S:C: ratio of total solanine to total chaconine, FW: Fresh weight											

Table 4.5c. Interaction effects of nitrogen (N) fertilisation x aphid infestation x cultivar on individual foliar glycoalkaloids (GAs) produced by five different groups of aphid infested (G2, G4) and non-infested (G1, G3, G5) potato plants cvs. King Edward (KE) and Maris Piper (MP)

Cultivars	Nutrient solutions	G1			G2			G3			G4			G5		
		sol	α -cha	S:C	α -sol	α -cha	S:C	α -sol	α -cha	S:C	α -sol	α -cha	S:C	α -sol	α -cha	S:C
KE	N ₂	47.8	86.0	36:64	30.5	69.3	27:73	91.5	178.2	34:66	41.5	76.7	35:65	126.0	243.6	34:66
KE	N ₄	33.7	60.5	36:64	13.2	22.2	37:63	33.5	66.5	34:66	18.7	33.1	36:64	41.5	82.9	33:67
MP	N ₂	25.3	33.1	43:57	19.8	25.8	43:57	43.1	65.8	40:60	16.1	20.1	45:55	68.8	100.6	42:58
MP	N ₄	6.8	10.1	38:62	8.3	10.6	44:56	14.1	21.6	40:60	10.1	13.1	43:57	31.9	47.6	40:60
SED		solanine: 6.3, chaconine: 9.3, S:C ratio: 0.055														
DF		60														
P		solanine: **, chaconine: ***, S:C ratio: NS														

SED: standard error of difference between means, DF: degrees of freedom

*** P<0.001, **P<0.01, NS: Non significant

Days: 0 15 45 60

G1:	-A			
G2:	-A	→	+A	
G3:	-A		-A	→
G4:	-A		+A	→
G5:	-A		-A	→

N₂: 100 mg total nitrogen/l solution, N₄: 300 mg total nitrogen/l solution

sol: total (α + β) solanine (mg/100g FW), cha: total (α + β) chaconine (mg/100g FW), S:C: ratio of total solanine to total chaconine, FW: Fresh weight

4.3.8 Aphid performance

Table 4.6a shows the effects of nitrogen fertilisation on the abundance of *M. persicae* after the infestation of potato plants cvs King Edward and Maris Piper grown in a low (N_2) and in a high (N_4) nitrogen solution. In general aphids which infested potato plants of both cultivars grown in the high nitrogen solution N_4 produced significantly more ($P<0.001$) offspring than those produced by aphids which infested plants grown in the low nitrogen solution N_2 (Table 4.6a).

Table 4.6a. Effects of nitrogen fertilisation on the fecundity of *Myzus persicae* adults fed on potato plants cvs. King Edward (KE) and Maris Piper (MP) grown in two different nutrient solutions

Cultivars	Nutrient solutions	Mean number of aphids/cm ² upper leaves	Mean number of aphids/cm ² middle leaves	Mean number of aphids/cm ² lower leaves	Total aphid abundance
KE+MP	N_2	11.32	8.2	7.11	8.88
KE+MP	N_4	22.62	9.8	8.86	13.78
SED		2.12	1.42	1.13	0.62
DF		21	21	21	21
P		***	NS	NS	***

SED: standard error of difference between means, DF: degrees of freedom
 *** $P<0.001$, NS Non significant

When the aphid performance was examined individually for each potato cultivar and every nitrogen treatment, a significant ($P<0.05$) difference was observed due to the effects of nitrogen fertilisation (Table 4.6b). Aphids performed better on plants grown in high nitrogen levels (N_4) in both varieties. In particular aphids which infested potato plants cv. King Edward grown in N_4 nutrient solutions produced significantly more ($P<0.05$) offspring than those which were produced by aphids which infested plants grown in N_2 nutrient solutions. The difference in the aphid performance between N_2 and N_4 plants was highly significant ($P<0.001$) in potato cv. Maris Piper (Table 4.6b).

In addition no significant ($P>0.05$) differences were observed in the overall aphid performance between the two potato cultivars (Table 4.6c).

Table 4.6b. Interaction effects of nitrogen fertilisation x cultivar on the fecundity of *Myzus persicae* adults fed on potato plants cvs. King Edward (KE) and Maris Piper (MP) grown in two different nutrient solutions

Cultivars	Nutrient solutions	Mean number of aphids/cm ² upper leaves	Mean number of aphids/cm ² middle leaves	Mean number of aphids/cm ² lower leaves	Total aphid abundance
KE	N ₂	16.63	6.02	8.49	10.38
KE	N ₄	19.40	10.29	8.71	12.80
MP	N ₂	6.01	10.39	5.73	7.38
MP	N ₄	25.80	9.41	9.00	14.75
SED		3.0	2.0	1.5	1.11
DF		21	21	21	21
P		**	NS	NS	*

SED: standard error of difference between means, DF: degrees of freedom

** $P<0.01$, * $P<0.05$, NS Non significant

Table 4.6c. Effects of cultivar on the fecundity of *Myzus persicae* adults fed on potato plants cvs. King Edward (KE) and Maris Piper (MP) grown in two different nutrient solutions

Cultivars	Nutrient solutions	Mean number of aphids/cm ² upper leaves	Mean number of aphids/cm ² middle leaves	Mean number of aphids/cm ² lower leaves	Total aphid abundance
KE	N ₂ +N ₄	18.02	8.16	8.60	11.59
MP	N ₂ +N ₄	15.93	9.90	7.37	11.07
SED		2.12	1.42	1.13	0.62
DF		21	21	21	21
P		NS	NS	NS	NS

SED: standard error of difference between means, DF: degrees of freedom

NS Non significant

However when the aphid performance was examined individually for each potato cultivar and every nitrogen treatment (Table 4.6b), potato plants cv. King Edward grown in the low nitrogen treatment produced significantly more ($P<0.05$) offspring than those produced by cv. Maris Piper plants grown in the same nitrogen treatment. No significant differences were observed in the aphid performance between the two cultivars grown in the high nitrogen treatment N₄.

4.3.9 Stress and after stress effects induced by aphid infestation in both experiments

Figure 4.17 shows the effects of aphid infestation on the total foliar glycoalkaloid production of the potato plants, examined separately for each of the four nitrogen levels applied (Exp. 1). In all nitrogen treatments the curve indicating the glycoalkaloid values detected in aphid-infested plants lies below that indicating glycoalkaloid values from the non-infested plants.

This was expected since in aphid-infested plants the glycoalkaloid synthesis was reduced. However in the very low (N_1) and low (N_2) nitrogen treatments the curves indicating the glycoalkaloids detected in both aphid-infested and non-infested plants are concave downwards, while in the medium (N_3) and high (N_4) nitrogen treatments are concave upwards.

Figure 4.18 shows the effects of aphid infestation on the total foliar glycoalkaloid production of the potato plants, examined separately for each nitrogen level applied to potato cvs. King Edward and Maris Piper (Exp. 2). As in experiment 1 in both nitrogen treatments and for both cultivars the curve indicating the glycoalkaloid values detected in aphid-infested plants lies below that representing glycoalkaloid values from the non-infested plants.

The glycoalkaloid synthesis was reduced in the aphid-infested plants of both potato cultivars. In addition, the curves indicating the glycoalkaloids detected in both aphid-infested and non-infested plants are concave upwards for both potato cultivars and nitrogen levels applied.

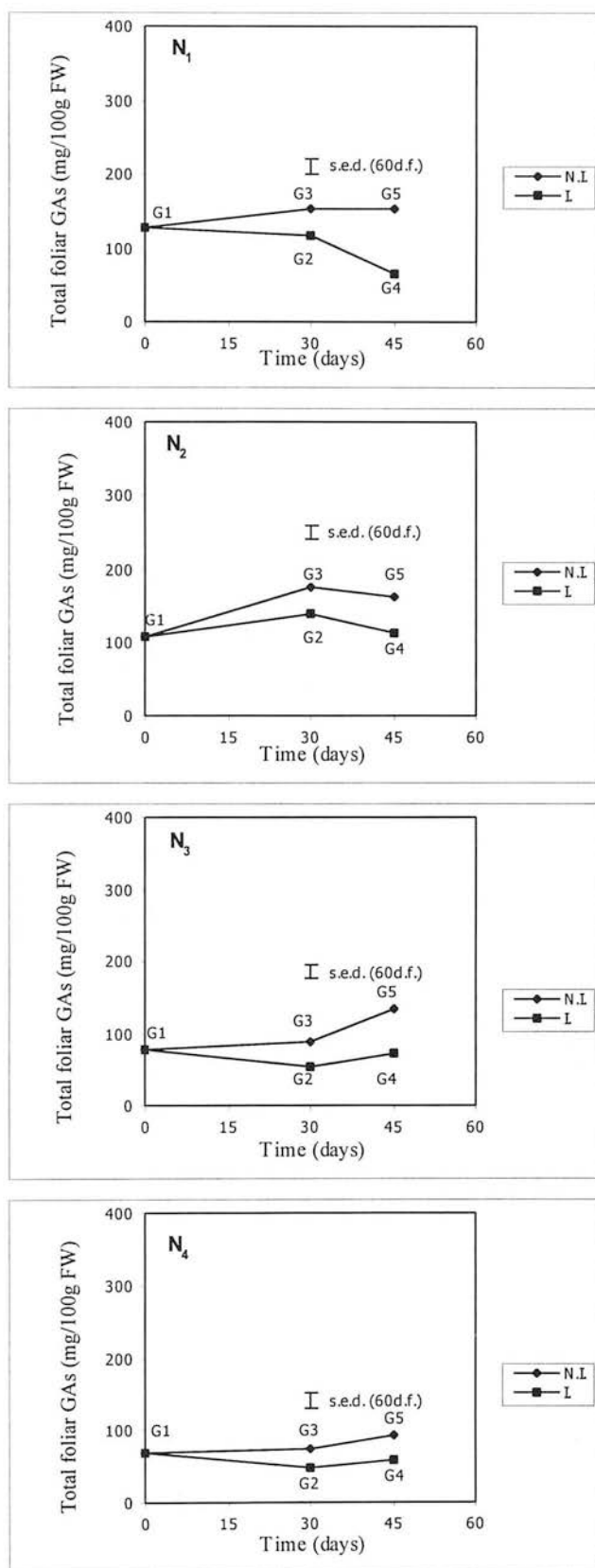


Figure 4.17. Total content (mg/100g) of foliar fresh (FW) weight glycoalkaloids (GAs) produced by aphid-infested (I.) and non-infested (N.I.) plants harvested at three different time intervals examined separately for each nitrogen level applied (Exp. 1).

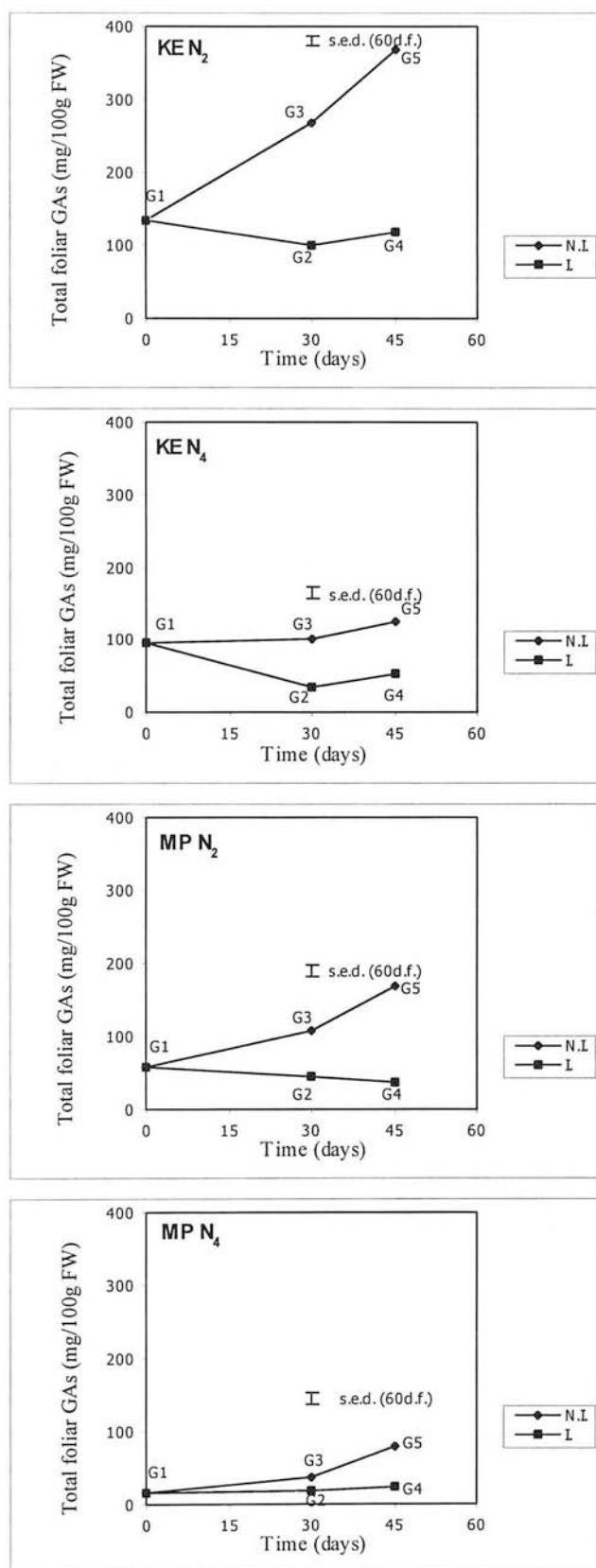


Figure 4.18. Total content (mg/100g) of foliar fresh (FW) weight glycoalkaloids (GAs) produced by aphid-infested (I.) and non-infested (N.I.) plants harvested at three different time intervals examined separately for each nitrogen level applied in potato cvs. King Edward and Maris Piper (Exp. 2).

Tables 4.7a and 4.7b show the stress and after stress effects on foliar glycoalkaloid production of potato plants due to aphid infestation in exp. 1 and exp. 2 for all of the nitrogen fertilisation levels applied. Across all nitrogen levels, aphids induced a reduction in the glycoalkaloid content produced during the period in which the plants were infested.

Table 4.7a. Stress and after stress effects of aphid infestation on the fresh weight total foliar glycoalkaloid (GA) production of potato plants cv. King Edward (Exp.1)

Nutrient solutions	Fresh weight GAs (mg/100g)	
	% stress	% after stress
N ₁	-23.6	-34.1
N ₂	-20.5	-9.2
N ₃	-38.4	-20.8
N ₄	-36.3	-7.2

N₁: 50 mg total nitrogen/l solution, N₂: 100 mg total nitrogen/l solution, N₃: 200 mg total nitrogen/l solution, N₄: 300 mg total nitrogen/l solution

Table 4.7b. Stress and after stress effects of aphid infestation on the fresh weight total foliar glycoalkaloid (GA) production of potato plants cvs. King Edward (KE) and Maris Piper (MP) (Exp.2)

Nutrient solutions		Fresh weight GAs (mg/100g)	
		% stress	% after stress
KE	N ₂	-63.0	-22.1
KE	N ₄	-64.5	-6.6
MP	N ₂	-58.1	-41.3
MP	N ₄	-47.2	-49.6

N₂: 100 mg total nitrogen/l solution, N₄: 300 mg total nitrogen/l solution

This is indicated by the negative percent values obtained for both stress and after stress effects. Removal of the aphids from the plants in most of the cases appeared to start reversing the situation by increasing the amounts of glycoalkaloids produced. However two exceptions were observed, one in potato cv King Edward in the very low N₁ nitrogen solution and a second in potato cv. Maris Piper in the high N₄ nitrogen solution where after stress effects appeared to be higher compared to stress effects of aphid infestation.

4.4 Discussion

In both experiments, aphid-infested plants were subjected to dense aphid colonisation. This resulted in a reduction of the amounts of green matter produced by aphid-infested potato plants compared to the amounts produced by non-infested plants at all nitrogen fertilisation levels applied to both potato cultivars. It has been reported that even mass feeding by aphids may cause insidious rather than obvious damage expressed as an overall reduction of growth of the plant (Dixon, 1971; Dixon, 1971; van Emden *et al.*, 1969; Ortman & Painter, 1960). Large numbers of phloem feeding aphids may reduce yields simply by removing enough photosynthate to stunt plant growth.

It has been suggested (Kennedy & Stroyan, 1959) that aphid feeding can temporarily increase the nutritional quality of the substrate. This occurs since it is believed that aphids are acting as “sinks” competing with the natural sinks of the plant (buds, flowers, or other growing parts) for nutritive substances (Hill, 1962). The plant is trying to assimilate this sink by increasing the metabolic rates of nutrient mobilisation and transportation in cells neighbouring the infested area (Petitt & Smilowitz, 1982; Dixon & Wratten, 1971). This results in a simultaneous reduction of photosynthesis in the affected tissues, chloroplast degeneration and varying degrees of chlorosis (Kantack & Dahms, 1957; Diehl & Chatters, 1956).

Glycoalkaloid levels of aphid-infested plants were found to be reduced not only as an immediate and direct effect of the dense colonisation of the plants, but also as an after-effect which followed the elimination of the aphids from the plants. These results are in agreement with those obtained by Zullo *et al.* (1984), who reported decreased foliar glycoalkaloid levels in *Solanum americanum* plants infested by the aphid *Aphis fabae* ssp. *solanella*. Data obtained from the first experiment of this chapter showed that, depending on the nitrogen fertilisation treatment, the glycoalkaloid reduction of potato cv. King Edward aphid-infested plants ranged between 20.5 to 38.4 % (Table 4.7a). In addition it appeared that the reduction of

glycoalkaloid levels in infested plants was increasing with nitrogen fertilisation. However the glycoalkaloid reduction observed in the second experiment for potato cv. King Edward was higher and stable ranging between 63 to 64.5 % (Table 4.7b). By contrast for potato cv. Maris Piper the stress effect was reduced approximately 10% from low (N_2) to high (N_4) nitrogen treatments. Both experiments showed that across most nitrogen treatments the glycoalkaloid reduction due to the after effects of aphid infestation ranged between 20.8 to 49.6 %. Two exceptions were observed in the low N_2 and the high N_4 nitrogen treatments of the first experiment and one in the high N_4 nitrogen treatment applied to the potato cv. King Edward where the after effects of aphid infestation induced a glycoalkaloid reduction of 6.6 to 9.2 %.

It has been reported (Osman & Zacharius, 1979) that the *in vitro* glycoalkaloid synthesis of γ -, β -, and α - forms of solanine and chaconine occurs when the aglycone steroid solanidine molecule is glucosylated in a stepwise synthesis with one, two and three sugars in its glycosidic part. The presence of the soluble sugars that exist in the glycoalkaloid molecule in sufficient quantities during the final steps of glycoalkaloid formation may play a crucial role in the amounts of glycoalkaloids that will finally be synthesised and expressed. Aphids interfere with photosynthetic efficiency in the infested plant and provide a rich medium for fungal growth through their excretion products (Schepers, 1989). Due to the normally low nitrogen/carbon ratio of phloem sap, aphids also have to adapt to a nutrient source with a suboptimal supply of nitrogen during much of the growing season. To extract enough usable nitrogen and acquire sufficient protein, aphids eliminate large quantities of phloem sap and excrete carbohydrate-rich honeydew (Llewellyn *et al.*, 1974; Mittler, 1958). Hence it is possible that lack of glucose, rhamnose and galactose -which make up the sugar part of the glycoalkaloid molecule- resulting from both photosynthesis rate reduction and carbohydrate absorbance by the aphids, led to a reduction in the amount of glycoalkaloids detected in the potato foliage.

Glycoalkaloid synthesis in tubers is greatly increased after wounding (Olsson, 1986). Apparently the physiological activity after wounding is enhanced in cells nearest the

wound. There is some circumstantial evidence to suggest that damage of the potato tops by hail or summer frosts increases the incidence of solanine in the Netted Gem cultivar (Hutchinson & Hilton, 1955). During stylet penetration which is usually considered to be intercellular, some cell walls are grazed or ruptured (Spiller *et al.*, 1985; Kimmins & Tjallingii, 1985) and some cells are killed (Pollard, 1973; Davidson, 1923); such mechanical damage has potential to cause wound reactions (Ryan *et al.*, 1981). Defensive reactions observed in wounded tissue of various plants can include the accumulation of secondary defensive substances such as tannins and their phenolic precursors (Puritch, 1977), triterpenes (Caputo *et al.*, 1979; Monaco *et al.*, 1974), and phytoalexins (Argandoña *et al.*, 1983; Nielson & Don, 1974; Puritch & Nijholt, 1974). It has been reported that one of the responses of plants to the feeding of sucking insects is the degeneration and disappearance of chloroplasts in the vicinity of the feeding puncture (Miles, 1989; Pollard, 1973). In addition aphids can induce photosynthetic stress at the level of the chloroplast that is transmitted across cell walls and plasma membranes. The limitation in photosynthetic rate can be observed while other cellular components like mitochondria, plasma or vacuolar membranes are damaged. This damage leads to cell death and therefore chloroplast death. Furthermore it has been suggested that the synthesis of solanidine occurs in the plastids (Ramaswamy *et al.*, 1976). Possibly the reduction in the foliar glycoalkaloid levels observed derived from a general reduction of solanidine following the reduction in the number of undamaged plastids occurring in the cells of the potato foliage.

In general, the amounts of glycoalkaloids produced by aphid-infested and non-infested potato plants of the cv. King Edward in the second experiment were increased compared to those produced in the first experiment by plants treated with the same nitrogen solutions (N_2 and N_4). The % stress effect observed in the second experiment was 2.7 and 1.7 times higher to that observed in the first for N_2 and N_4 treatments respectively. In addition, the curves indicating the glycoalkaloids detected in low (N_2) nitrogen treatments in both aphid-infested and non-infested plants are concave upwards in exp 2, while they are concave downwards in exp. 1.

This variation could be due to the fact that in the glasshouse the temperature and relative humidity conditions were fluctuating from day to night, while in the plant growth room they were not. This could alter not only the photosynthetic and nutritional physiology of potato plants but could also affect the infestation and population growth rates of the aphids. However comparison of results concerning the aphid infestation between the two experiments cannot be drawn. This is because in the first experiment the methods used for measuring the aphid performance (individual adult cumulative fecundity, intrinsic rate of increase) were different from those used in the second experiment (total aphid abundance).

An aphid that deposits honeydew on the leaf surface could reduce photosynthesis through stomatal limitation (Ryan *et al.*, 1990). The accumulation of honeydew can often promote the growth of saprophytic fungi, reduce photosynthesis and lead to the premature senescence of leaves independent of direct aphid feeding (Rabbinge *et al.*, 1981; Wratten, 1975; Bardner & Fletcher, 1974). The elevation of plant free amino acid content due to senescence may add to the number of leaves highly suitable for aphid feeding.

The elevated amounts of glycoalkaloids detected in potato tubers deriving from Colorado potato beetle infested plants (Hlywka *et al.*, 1994) were attributed to the aggressive chewing feeding behaviour of the insect. By contrast plants infested by potato leafhopper which is considered to be a phloem feeding insect produced normal amounts of tuber glycoalkaloids. However the general effect on the whole plant of the presence of many aphid sinks is a depletion of carbohydrates as shown for tobacco infested by *M. persicae* (Baron & Guthrie, 1960). It has been suggested (Kloft, 1960) that aphid infested plants show a decrease in photosynthesis and an increase in respiration, leading to a further depletion of carbohydrate in the plant. This depletion of carbohydrates following aphid infestation is clearly seen in the reduction of root yield (van Emden *et al.*, 1969; Ortman & Painter, 1960). Aphids may indirectly alter the nitrogen metabolism of their hosts by affecting root mass. Plants infested with aphids invariably suffer a reduction in root growth. The

hypothesis that peach potato aphid infested plants can produce elevated amounts of tuber glycoalkaloids indirectly by reducing root mass or by altering the carbohydrate and soluble nitrogen concentrations required for tuber formation could be the subject of future investigations.

The results from both glasshouse and plant growth room experiments suggest that heavy aphid infestation is negatively affecting the defensive system of the potato plant and that this negative effect is related to its secondary metabolism. This was obvious for both low and high levels of available nitrogen. However it appears that reduction of glycoalkaloid synthesis was more intense when nitrogen was applied at high or excessive quantities. Hence nitrogen availability plays a crucial role related to the general health status of the potato crop because as was proved in the previous chapter, it affects the glycoalkaloid concentration in the foliage. An implication of these results is that in organic farming where the nitrogen availability is restricted, plants are grown effectively protected from pests due to the presence of natural pesticides. It can be assumed that a potential aphid infestation would induce less damage to the natural defensive system of potato crop when grown in an organic production system. In addition in a conventional farming system where nitrogen is efficient if not in excess, plants could be more susceptible in terms of pest infestation because a reduction of natural defensive toxins could be observed.

Chapter 5

Effects of aphid infestation on the foliar glycoalkaloid production of potato, grown in the field

5.1 Introduction

The results of the previous chapter showed a reduction of total foliar glycoalkaloids resulting from the dense colonisation of aphid-infested plants. This was observed at all nitrogen levels applied and in both potato cultivars. Since these results were obtained under plant growth room or glasshouse conditions, it is important to examine if aphid-infested plants produce reduced amounts of foliar glycoalkaloids in the field. Reduced amounts of foliar glycoalkaloids after aphid infestation could imply an adverse effect on the natural defence system of potato which would possibly fail to protect the crop from further aphid infestations. Furthermore it is important to examine if the source of nitrogen fertilisation e.g. natural or synthetic plays any role in the foliar glycoalkaloid production of the aphid-infested or non-infested plants. Hellenas & Branzell (1995), suggested that studies at two sites in Sweden showed that potato tubers grown using manure had lower contents of α -solanine and α -chaconine than those grown with syntetic fertiliser.

Sustainable agriculture has been associated with minimising the use of nutrient inputs from outside as well as losses from within the system (Lampkin, 1990). The supply of nitrogen is considered as a principal factor limiting organic crop production. The experiments of chapter 3 showed that increasing nitrogen fertilisation reduced the glycoalkaloid content produced in potato foliage. In addition Stockdale *et al.* (1992), suggested that manure application increased the numbers of *M. euphorbiae* and *M. persicae* aphids on potatoes early in the cultivating season on potatoes. The aim of this chapter was to examine if potato plants grown with fertiliser produce higher content of foliar glycoalkaloids compared to plants grown with manure; an additional aim was to investigate if aphid infestation could reduce - similarly to plant growth room and glasshouse experiments- the amounts of foliar glycoalkaloids produced in potato cvs King Edward and Maris Piper grown in the field.

5.2 Materials and Methods

One experiment was conducted at Bush Estate Lothian Region Scotland. The potato varieties King Edward and Maris Piper which were used in the experiments of Chapters 3 and 4 were grown in pots between June-September 1996. In this experiment the potato plants were infested by the aphid *M. persicae*. The glycoalkaloids produced by the aphid-infested plants were compared to those produced by aphid-free plants.

5.2.1 Growing media

The potato plants were grown in pots containing soil. This derived from two neighbouring fields and was classified as poorly drained clay soil of Stirling Series (SG) (fluvisol in Food and Agriculture Organisation or fluvaquent in USDA classifications) (Laing, 1976). In the neighbouring fields different methods were used for cultivation. Organic methods of cultivation were used in the field managed by Jamesfield farm, Abernethy. The conventional to organic conversion started in 1985 and no lime had been added to the soil since 1983. Conventional methods of cultivation (adding of chemically prepared fertilisers and agricultural chemicals) were used in the field managed by Balgonie farm.

Topsoil (20-30cm) was sampled randomly at different sites from both fields, placed in labeled bags and transferred to Bush Estate for storage. To compare the manure to fertiliser treatments, the amounts of available nitrogen to the potato plants should be the same in both. Nitrogen could then be ignored as a variable between the manure and fertiliser treatments. For this reason the soil was analysed for pH, extractable ammonium N, nitrate N, phosphorus, potassium, magnesium and total nitrogen before sieving, mixing and homogenisation. A second analysis was performed before the starting date of the experiment. The analyses were performed at two different time points to indicate the approximate rate of nitrogen degradation in the two different soil samples. The soil and manure analyses as performed in Soil Science Department of SAC are presented in table 5.1.

The total available N in the soil derived from the organic farm was found to be less than that detected in the soil derived from the conventional farm (Table 5.1). The sheep manure which was derived from the organic farm was mixed, homogenised, and analysed for extractable ammonium N and total available N.

Table 5.1. Soil Analysis Report

	Jamesfield Organic		Balgonie Conventional		Farmyard Manure
pH	7.1		6.6		
Lime required arable	0 t/ha		0 t/ha		
Date of analysis	3/6	13/6	3/6	13/6	3/6
Extract. NH_4^+ -N (mg/kg)	28.5	15.1	47.7	23.2	610
Extract. NO_3^- -N (mg/kg)	81.5	82.5	91.1	97.7	
Total available N (mg/kg)	110	97.6	138.5	120.9	4130
Extract. P (mg/l)	100		43.8		
Extract. K (mg/l)	711		360		
Extract. Mg (mg/l)	248		179		

It was concluded that a small amount (approx. 13g/pot) of manure had to be added to the soil which was sampled from the organic farm (manure treatment) so that the total available nitrogen in both soils (derived from the organic and the conventional fields) would be the same.

5.2.2 Plant material

Potato tubers (*S. tuberosum* L. cvs. King Edward and Maris piper) were selected for approximately similar size and health condition. Pots (diameter: 23cm depth: 23cm) were stood on rectangular plastic trays (22cm x 16.5cm x 4.5cm) and made level with the soil surface, under open field conditions. Two different types of fertilisation treatments (fertiliser and manure) were combined with aphid-infested and non-infested plants. The pots were filled with soil or soil + manure up to 2/3 of their volume and covered with soil after the tuber had been planted and watered. The potato plants were divided as in the experiments of Chapter 4, into five groups representing aphid-infested and non-infested plants harvested on different dates.

The aphids used in this experiment derived from the laboratory clone culture of the

aphid *M. persicae*, which was established in the insectary (Chapter 2). Thirty standardised adult aphids per plant (approximately five/leaf) were transferred from the basic culture (Chinese cabbage) to the treatments subjected to infestation. Plants were infested with aphids when they had reached a predetermined developmental stage (full development of the second true leaf) approximately 20 days after planting. In particular, the five different groups of aphid-infested and non-infested plants were:

Group 1, (G1): Non aphid-infested plants harvested after 20 days from the effective starting date of the experiment which is considered to be the first watering with nutrient solutions.

Group 2, (G2): Aphid-infested plants from 20 days after the effective starting date of the experiment and harvested after 40 days from infestation.

Group 3, (G3): Non aphid-infested plants harvested after 40 days from the effective starting date of the experiment.

Group 4, (G4): Aphid-infested plants from 20 days after the effective starting date of the experiment, aphids killed after 20 days from infestation and plants harvested 20 days after removal of aphid infestation.

Group 5, (G5): Non aphid-infested plants harvested after 60 days from the effective starting date of the experiment.

A schematic representation of the aphid infestation treatments applied to the potato plants and times of harvesting is given in graphic representation 5.1:

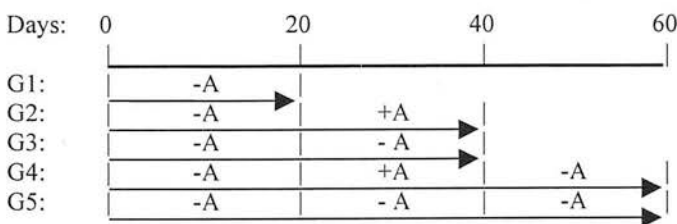


Figure 5.1. Schematic representation of the *Myzus persicae* infestation groups (G1, G2, G3, G4 and G5) applied to the potato plants plotted against times of harvesting; +A: aphid-infested period, -A: aphid-free period.

The extermination of the aphids in the aphid-infested G2 and G4 plants was achieved

by spraying the plants with soft soap solution 0.5 %. Sample preparation, extraction, purification and HPLC analysis were performed as previously outlined in Chapter 3.

5.2.3 Assessment of aphid performance

The aphid performance was measured using two methods. The first is the method of the intrinsic rate of natural increase already described in chapters 2 and 4. The second was a modification of the method of total aphid abundance as already described in chapter 4. For measuring of the intrinsic rate of natural increase, aphids were confined – as in the first experiment of the previous chapter - to leaves with leaf clip-on cages. Two standardised apterous pre-reproductive adult aphids were caged on the second true leaf to reproduce for 24 hours. The cage was placed on the abaxial surface of the leaf. After this period the adults were removed and the 3-5 new born nymphs were monitored daily. The nymphs were left to approach the 4th instar stage and, finally, two were allowed to remain in the clip cage and develop in to apterous pre-reproductive adults, the others being discarded. The pre-reproductive adults and their clip cage were transferred to a younger leaf to minimise damage to plants. This transfer was repeated every 6 days after the retained aphids reached maturity. The time taken to mature and the number of offspring produced were recorded. Offspring were counted and removed during the afternoon of the first day of reproduction and then every second day thereafter for a total period of the same number of days as had been needed to develop from 1st instar to reproductive adult. The intrinsic rate of natural increase (r_m) was then calculated for each clip-cage of aphids as in Chapters 2 and 4 according to the formula given by Wyatt & White (1977):

$$r_m = 0.74((\ln M_d)/d)$$

where d is the pre-reproductive time in days (from birth to first reproduction), and M_d is the number of progeny produced in the ensuing period of length d .

The aim of the experiments presented in this chapter was to measure the levels of glycoalkaloids produced by plants suffering from heavy aphid infestation and

compare them to the levels produced by non-infested plants. To achieve this target a dense colonisation of the plants by aphids was required. For this reason the aphids were left to colonise the potato plants for a total period of one month. However in field conditions the aphid colony did not thrive owing to extensive predation and parasitism, added to the low average temperatures observed during the growing period. Hence dense aphid colonisation did not occur and the plants of G2 and G3 groups were harvested 10 days earlier than it had been planned initially (20 days after infestation) shortening the duration of the experiment. For the measurement of aphid abundance, aphids were placed on the first true leaf of the potato plants and left to reproduce. The aphid infested leaf was covered by a pocket made from fine nylon mesh for protection from aphid parasitoids and predators. After twenty days the aphids –adults and nymphs- present on each leaf were counted using a hand-counter. After the measurement the aphids were brushed away and the leaf area was measured using a LI-COR leaf area meter model 3100 (Lambda Instruments Corporation, Lincoln Nebraska, USA). The aphid abundance was calculated as the number of aphids per cm² of leaf. The same method was applied to potato plants of the G4 group. Each excised leaf of this group was placed after the aphid measurement in a labeled paper envelope and stored in the deep freeze (-80°C) for further glycoalkaloid analysis. For the same purpose one leaf was excised from every non-infested plant of G5 group, placed in a labeled paper envelope and stored in the deep freeze.

From a statistical perspective the experiment was a replicated three factor experiment with plants grown in soil deriving from the organic and the conventional fields, 5 levels of aphid/harvest (G1, G2, G3, G4 and G5), and two potato cultivars King Edward and Maris Piper. The treatment combinations were repeated four times. In total 80 pots were placed in the field. Pots were randomly relocated every second day. The replicates were uniformly dispersed. In some plots the tubers did not sprout, and in some others the plants were seriously damaged from summer hail during the experiment. In total seven plots were missing during harvesting. The data were analysed using the General Linear Model procedures of MINITAB® (vs.11.1). It was necessary to use this procedure rather than the “Analysis of Variance” in order

to deal with the missing values. The output of this procedure was an analysis of variance, plus tables of means with standard errors. The differences between means were measured by t-test.

5.3 Results

5.3.1 Effects of fertilisation, cultivar and aphid infestation on green and dry matter

Table 5.2a shows the effects of fertilisation x cultivar on the green and dry matter produced by aphid infested and non-infested potato plants. These were not significant ($P>0.05$).

Table 5.2a. Interaction effects of fertilisation x cultivar on the green (FW) and dry matter (DW/FW) produced by potato plants cvs. King Edward (KE) and Maris Piper (MP)

Cultivar	Fertilisation	Green matter FW (g)	Dry matter (DW/FW)
KE	Manure	42.95	0.108
KE	Fertiliser	59.27	0.111
MP	Manure	46.21	0.106
MP	Fertiliser	58.38	0.120
SED		3.34	0.002
DF		53	53
P		NS	NS

SED: standard error of difference between means, DF: degrees of freedom
 NS: Non significant
 FW: fresh weight, DW: dry weight

However there was a highly significant ($P<0.001$) overall effect of the type of fertilisation on the amounts of green matter produced by both varieties. In particular plants from potato cv. King Edward treated with manure produced significantly ($P<0.001$) less amounts of green matter compared to the amounts produced by plants grown in soil subjected to nitrogen fertilisation. This was observed as well for potato cv. Maris Piper.

No significant ($P>0.05$) differences were observed between the overall amounts of dry matter produced by potato cv. King Edward. By contrast potato cv. Maris Piper

plants treated with manure produced significantly ($P < 0.001$) less dry matter than the level produced by fertiliser-treated plants. As for the green matter the interaction effect of fertilisation by cultivar on the dry matter produced was not significant ($P > 0.05$).

The significant ($P < 0.01$) effect of fertilisation by aphid infestation on the green matter observed (Table 5.2b) was mainly due to the reduced amounts of green matter produced in the G1 treatments. However no significant ($P > 0.05$) differences were observed between aphid-infested and non-infested plants from both varieties (KE+MP) harvested after 30 and 45 days from aphid infestation (G2 vs G3 and G4 vs G5 respectively). This result was observed for both manure- and fertiliser-treated plants.

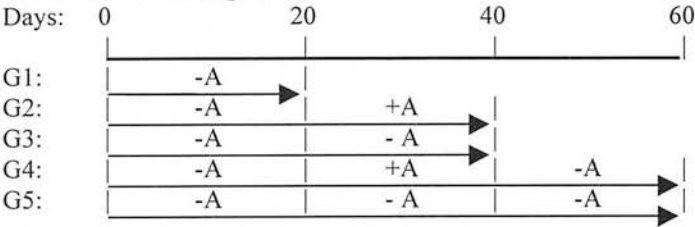
No significant ($P > 0.05$) effect due to aphid infestation was observed in the amounts of dry matter produced by aphid-infested (G2 and G4) and non-infested (G3 and G5) plants of both varieties (KE+MP) which were treated with manure or fertiliser. No significant differences ($P > 0.05$) were observed in the green and dry matter levels between manure- and fertiliser-treated plants in the non-infested plants harvested in the G1 group for both potato cultivars (KE & MP) (Table 5.2c). In addition there was no significant ($P > 0.05$) interaction between type of fertilisation, aphid infestation and cultivar on the green and dry matter produced by both potato cultivars (Table 5.2c).

Table 5.2b. Interaction effects of fertilisation x aphid infestation on the green (FW) and dry matter (DW/FW) produced by aphid infested (G2, G4) and non-infested (G1, G3, G5) potato plants cvs. King Edward (KE) and Maris Piper (MP)

Fertilisation		Green matter FW (g)					Dry matter (DW/FW)				
		G1	G2	G3	G4	G5	G1	G2	G3	G4	G5
KE+MP	M	41.20	57.50	50.66	36.54	37.01	0.111	0.108	0.104	0.116	0.109
KE+MP	F	38.54	62.24	68.54	61.52	63.28	0.117	0.109	0.112	0.118	0.121
SED				5.28					0.004		
DF				53					53		
P				**					NS		

SED: standard error of difference between means, DF: degrees of freedom

** P<0.01, NS: Non significant



M: Manure, F:Fertiliser

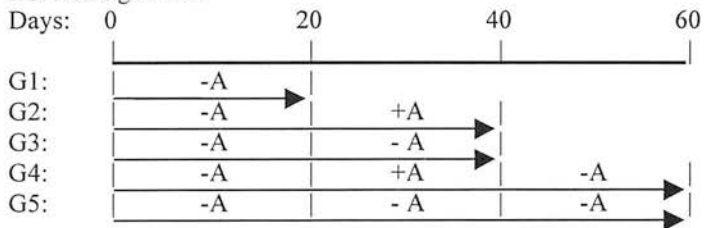
FW: fresh weight, DW: dry weight

Table 5.2c. Interaction effects of fertilisation x aphid infestation x cultivar on the green (FW) and dry matter (DW/FW) produced by aphid infested (G2, G4) and non-infested (G1, G3, G5) potato plants cvs. King Edward (KE) and Maris Piper (MP)

Fertilisation		Green matter FW (g)					Dry matter (DW/FW)				
		G1	G2	G3	G4	G5	G1	G2	G3	G4	G5
KE	M	47.75	52.82	48.14	34.06	31.99	0.103	0.106	0.105	0.117	0.109
KE	F	43.16	63.80	65.91	63.71	59.76	0.106	0.104	0.107	0.117	0.121
MP	M	34.65	62.17	53.17	39.02	42.04	0.118	0.109	0.102	0.114	0.109
MP	F	33.93	60.67	71.18	59.33	66.80	0.128	0.114	0.118	0.119	0.120
SED		7.47					0.005				
DF		53					53				
P		NS					NS				

SED: standard error of difference between means, DF: degrees of freedom

NS: Non significant



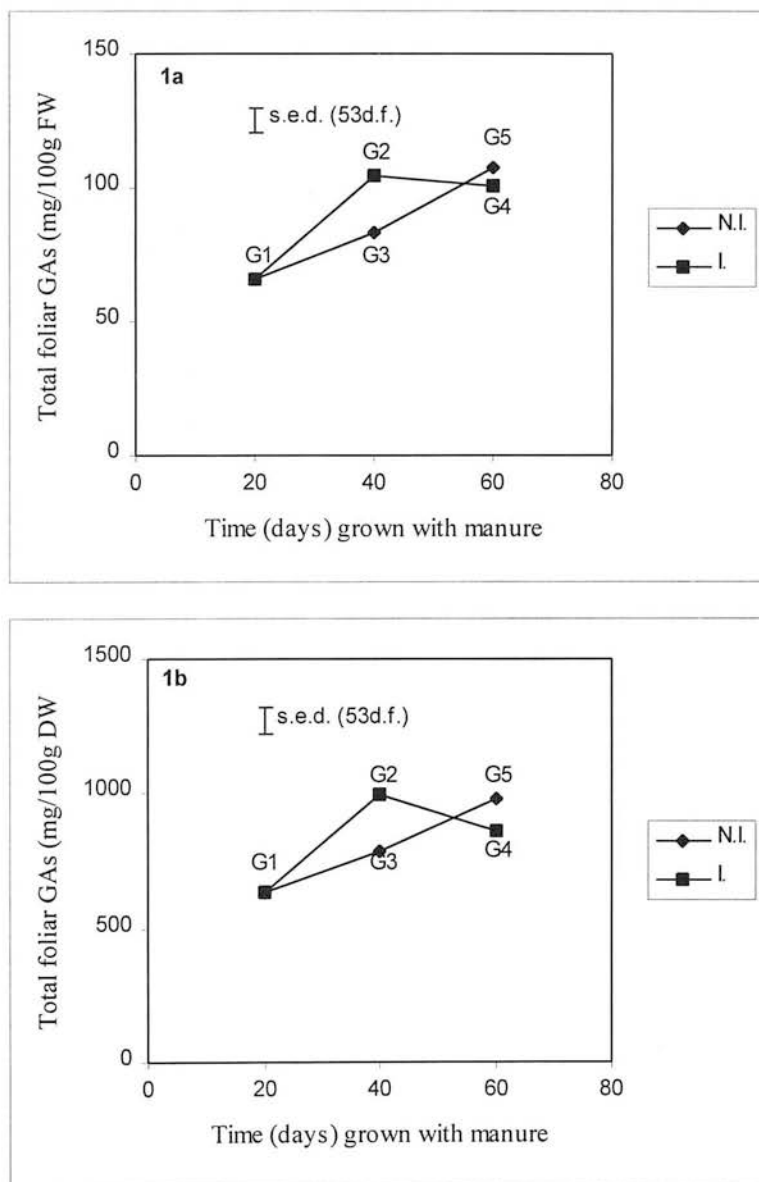
M: Manure, F: Fertiliser

FW: fresh weight, DW: dry weight

5.3.2. Effects of fertilisation and aphid infestation on total glycoalkaloid production

Potato cv. King Edward

Figures 5.1a and 5.1b show the effects of aphid infestation on the total foliar fresh and dry weight glycoalkaloid production of potato cv. King Edward plants grown with manure.



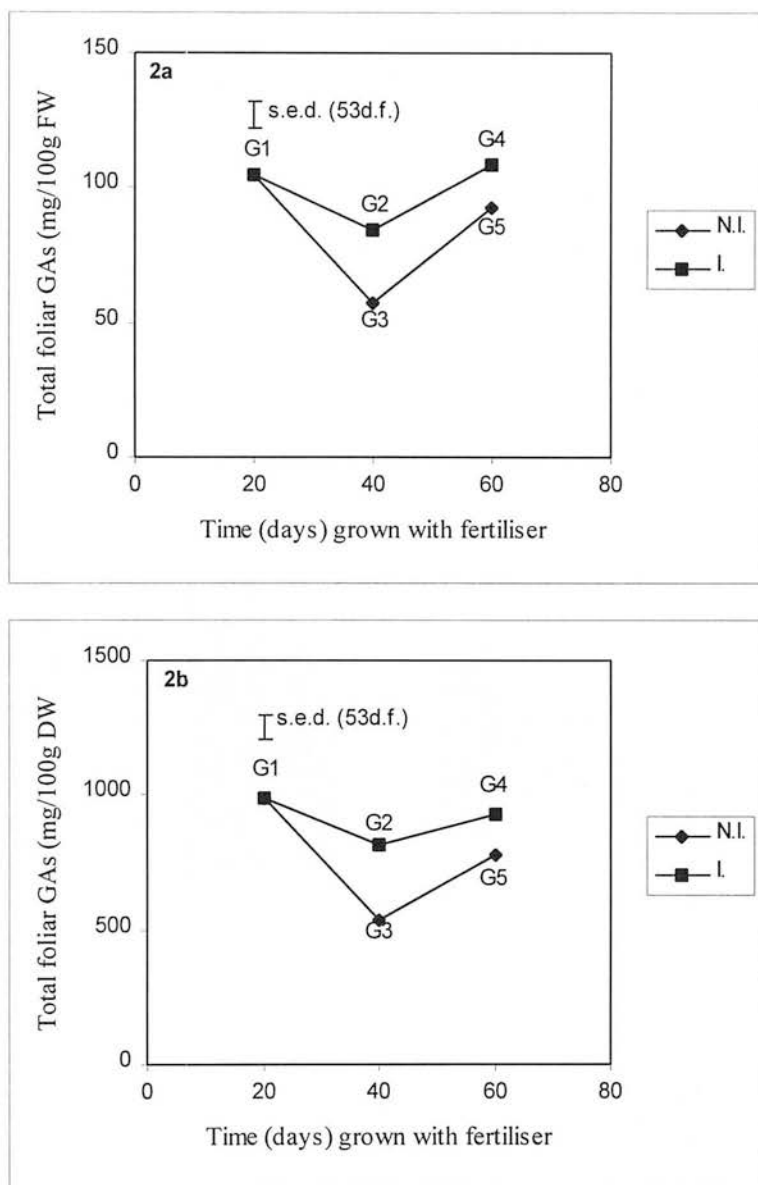
Figures 5.1a & 5.1b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids (GAs) produced by manure grown, aphid-infested (I. - G2 & G4) and non-infested (N.I. - G1, G3 & G5) potato plants cv. King Edward, harvested at three different time intervals.

Aphid-infested plants of G2 treatment produced significantly more ($P < 0.05$) foliar fresh and dry weight glycoalkaloids compared to those produced by the non-infested plants of G3 treatment. However no significant differences ($P > 0.05$) were observed between aphid-infested and non-infested plants harvested 20 days later (G4 and G5 treatments).

Time of harvesting increased the amounts of total foliar fresh or dry weight glycoalkaloids produced by the non-infested manure grown plants. Plants harvested 20 days after the starting date of the experiment (G1 treatment) produced less – although not significant ($P > 0.05$) – fresh or dry weight glycoalkaloids compared to non-infested plants harvested 20 days later (G3 treatment). In addition the G3 plants produced significantly ($P < 0.05$) less fresh or dry weight glycoalkaloids compared to the amounts produced by G5 treatment. The difference between G1 and G5 plants was highly significant ($P < 0.001$). However time of harvesting did not induce any significant effect ($P > 0.05$) on the total amounts of fresh or dry weight foliar glycoalkaloids produced by aphid-infested plants (G2 and G4 treatments, Figures 5.1a & 5.1b).

Similar results were observed in the fertiliser grown potato plants (Figures 5.2a and 5.2b) where the aphid-infested plants G2 produced significantly ($P < 0.01$) more glycoalkaloids compared to the non-infested plants G3. As for the manure-treated plants, no significant ($P > 0.05$) difference was observed in the amounts of glycoalkaloids produced between G4 and G5 treatments.

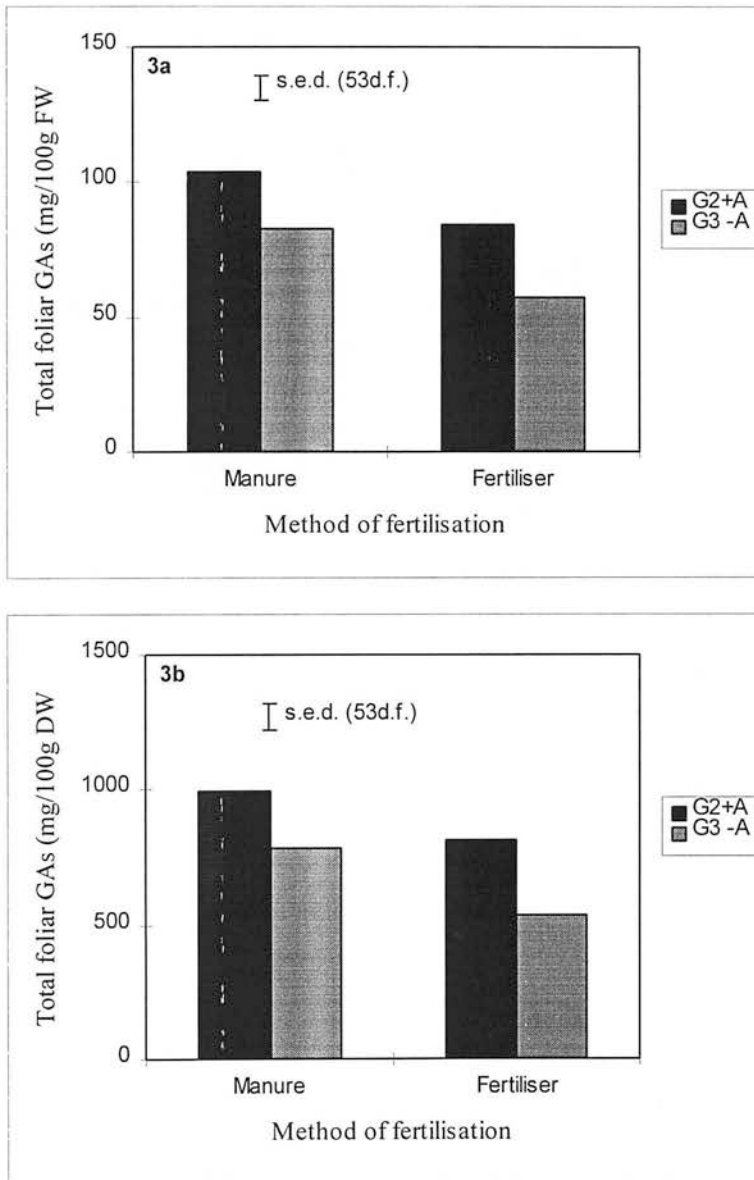
By contrast to the results obtained from the plants grown with manure, time of harvesting reduced the amounts of fresh or dry weight glycoalkaloids produced. In particular non - infested plants harvested 40 days after the starting date of the experiment (G3 treatment) produced significantly ($P < 0.001$) less fresh weight glycoalkaloids to those produced by G1 and G5 treatments (Figures 5.2a & 5.2b).



Figures 5.2a & 5.2b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids (GAs) produced by fertiliser grown, aphid-infested (I. - G2 & G4) and non-infested (N.I. - G1, G3 & G5) potato plants cv. King Edward, harvested at three different time intervals.

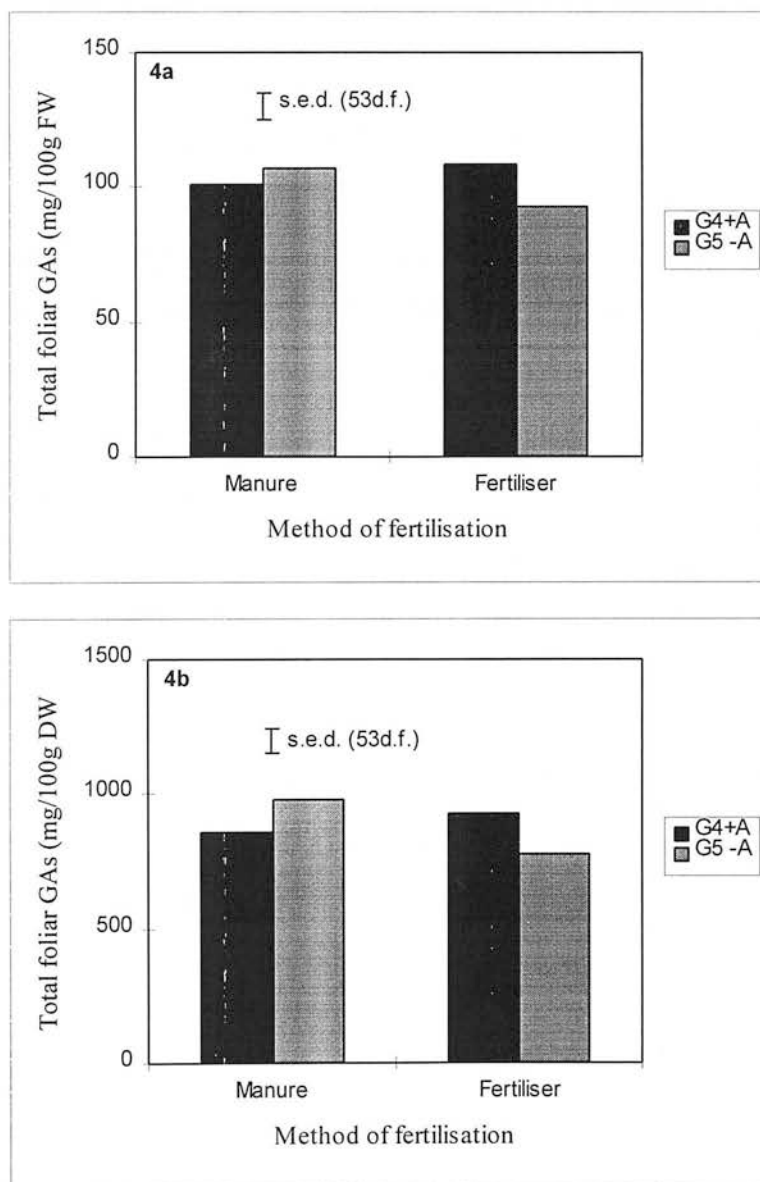
However no significant ($P > 0.05$) differences were observed in the amounts of foliar glycoalkaloids produced between G1 and G5 treatments. Aphid-infested plants harvested 20 days after the aphid infestation (G2 treatment) produced significantly less ($P < 0.05$) fresh weight glycoalkaloids to those produced by aphid-infested plants harvested at the end of the experiment (G4 treatment). However this reduction was not significant ($P > 0.05$) when the glycoalkaloid production was expressed on a dry weight basis.

Figures 5.3a and 5.3b show the effects of fertilisation method on the total fresh and dry weight glycoalkaloid production of aphid-infested and non-infested potato cv. King Edward plants harvested 20 days after aphid infestation. For both manure- and fertiliser-treated plants aphid infestation increased significantly ($P<0.05$ and $P<0.01$ respectively) the amounts of total fresh and dry weight foliar glycoalkaloids produced.



Figures 5.3a & 5.3b Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids (GAs) produced by aphid-infested (G2) and non-infested (G3) potato plants cv. King Edward grown under manure and nitrogen fertilisation.

Manure-treated plants infested by aphids (G2 treatment) produced significantly ($P<0.05$) more total foliar fresh weight glycoalkaloids compared to those produced by fertiliser-treated plants. However this difference was not significant ($P>0.05$) when the glycoalkaloid production was expressed as mg produced per 100g dry weight. In addition manure-treated non aphid-infested plants (G3 treatment) produced significantly ($P<0.01$) more foliar fresh or dry weight glycoalkaloids compared to those produced by plants treated with fertiliser.



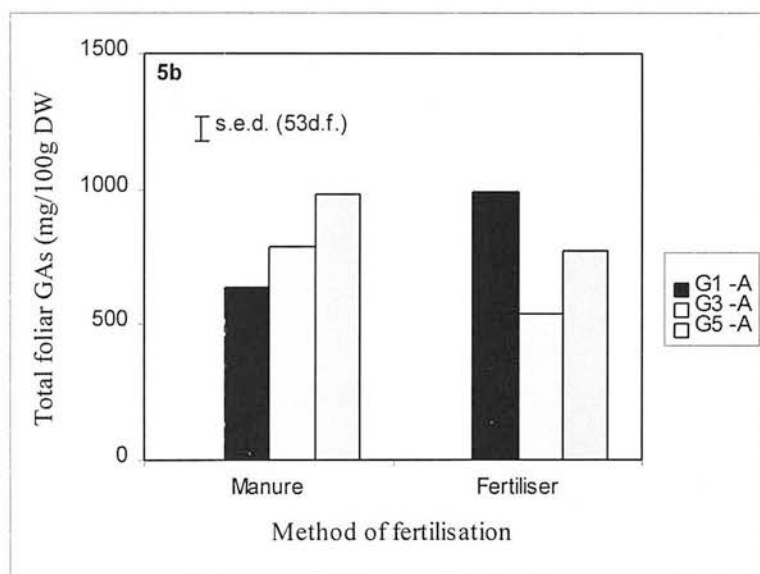
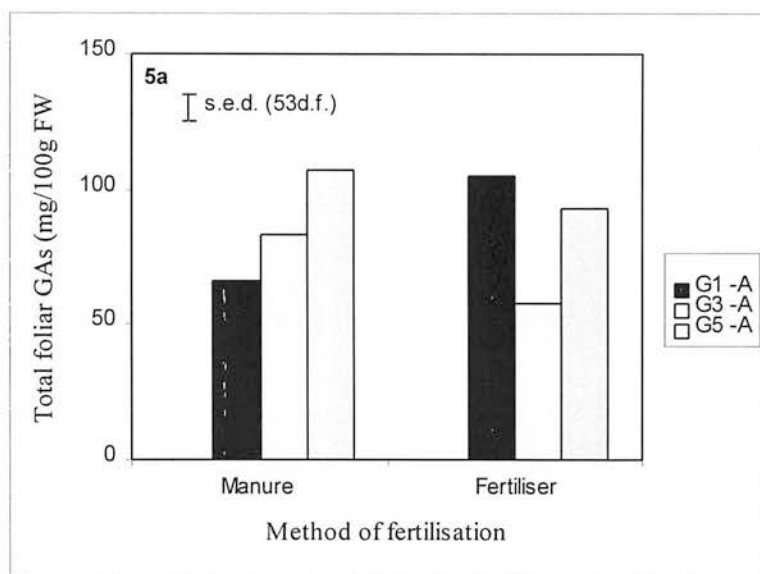
Figures 5.4a & 5.4b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids (GAs) produced by aphid-infested (G4) and non-infested (G5) potato plants cv. King Edward grown under manure and nitrogen fertilisation.

No significant differences ($P>0.05$) were observed in the total foliar fresh or dry weight glycoalkaloid production, between aphid-infested and non-infested plants (G4 and G5 treatments) grown with manure or fertiliser and harvested 40 days after the aphid infestation (Figures 5.4a and 5.4b).

In addition, the difference in the fresh or dry weight foliar glycoalkaloids produced by aphid-infested plants (G4 treatment), grown either with manure or fertiliser was not significant ($P>0.05$). Non aphid-infested plants of G5 treatment grown with manure produced significantly more ($P<0.05$) dry weight foliar glycoalkaloids to those produced by plants grown with fertiliser. This difference was not significant ($P>0.05$) when the glycoalkaloid production was expressed on a fresh weight basis.

Manure-treated plants harvested 20 days after the starting date of the experiment (G1 treatment) produced significantly ($P<0.001$) less foliar fresh or dry weight glycoalkaloids compared to the amounts produced by those plants grown with fertiliser (Figures 5.5a & 5.5b). However this was reversed in plants harvested 20 days later (G3 treatment) when fertiliser-treated plants produced significantly ($P<0.01$) less fresh or dry weight glycoalkaloids compared to those produced by plants treated with manure.

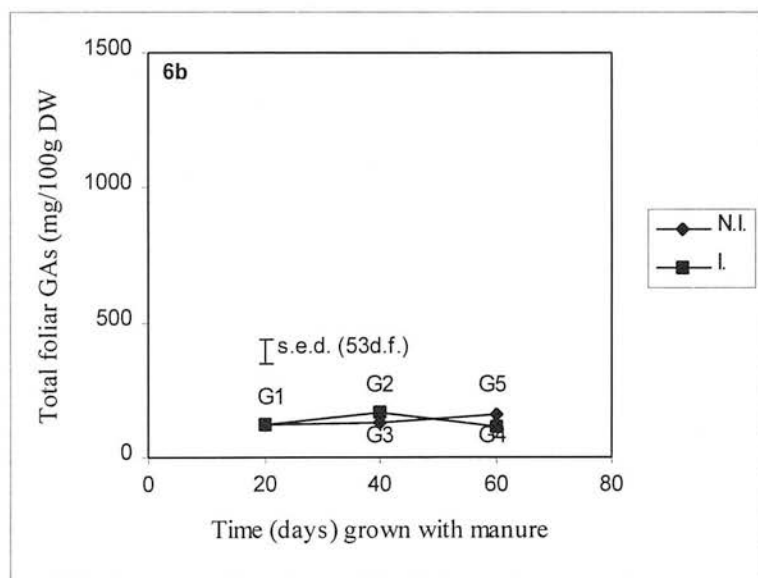
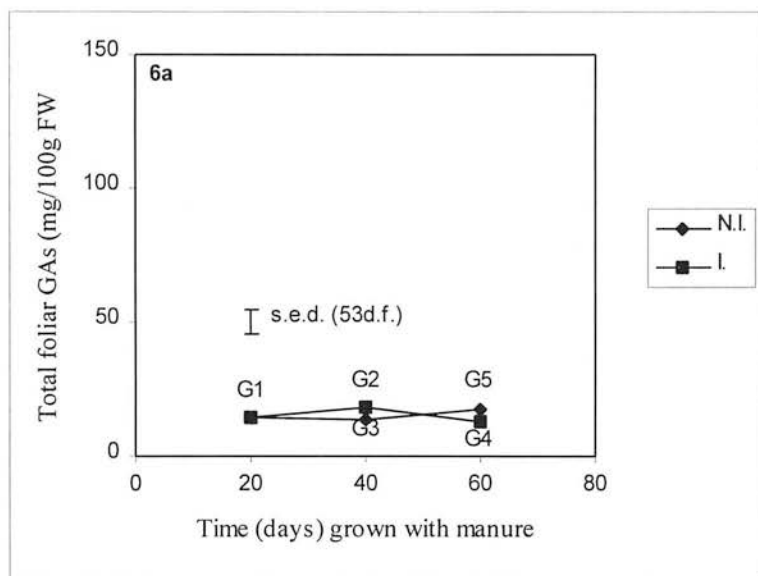
Manure-treated plants harvested at the end of the experiment (G5 treatment) produced more –although not significant ($P>0.05$)- fresh weight glycoalkaloids to those produced by fertiliser-treated plants. However this difference was significant ($P<0.05$) when the glycoalkaloid production was expressed on a dry weight basis.



Figures 5.5a & 5.5b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids (GAs) produced by non-infested potato plants cv. King Edward, grown under manure and nitrogen fertilisation and harvested at three different developmental stages (G1, G3 and G5).

Potato cv. Maris Piper

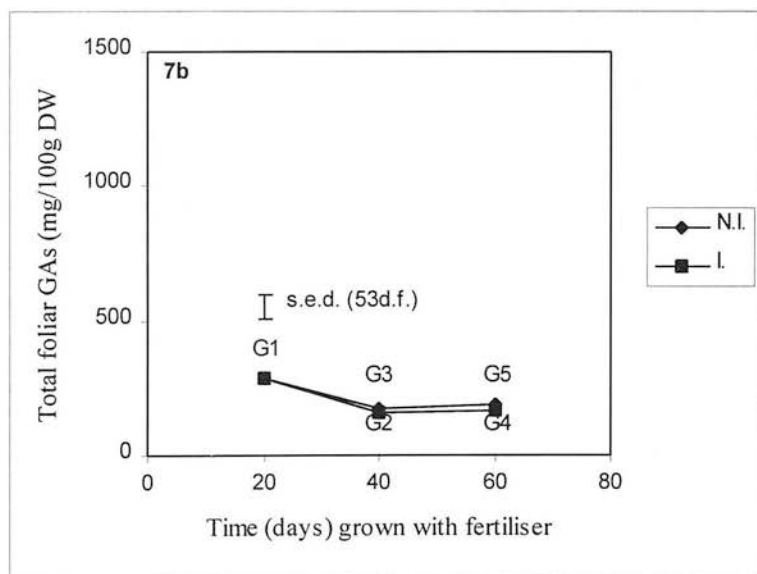
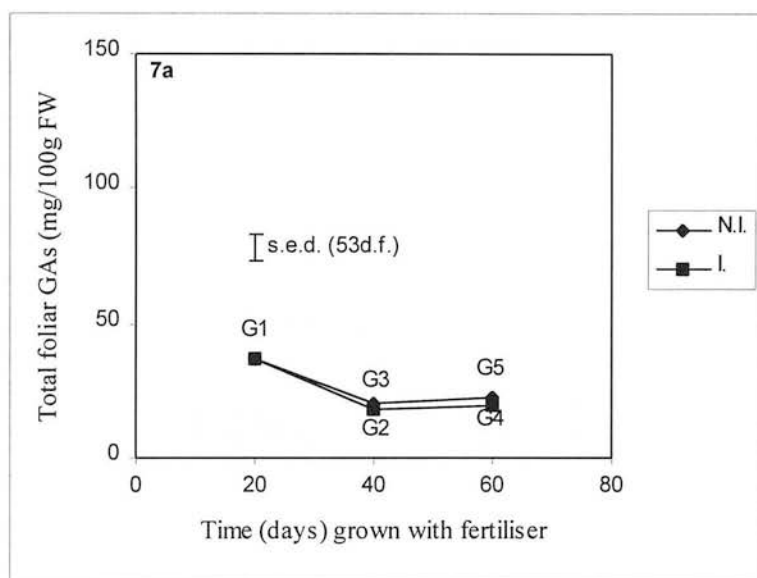
Figures 5.6a and 5.6b show the effects of aphid infestation on the total foliar fresh and dry weight glycoalkaloid production of potato cv. Maris Piper plants grown with manure. No significant ($P > 0.05$) effects on the total foliar fresh or dry weight glycoalkaloid production were observed due to aphid infestation of plants harvested 40 and 60 days after the starting date of the experiment.



Figures 5.6a & 5.6b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids (GAs) produced by manure grown, aphid-infested (I. - G2 & G4) and non-infested (N.I. - G1, G3 & G5) potato plants cv. Maris Piper, harvested at three different time intervals.

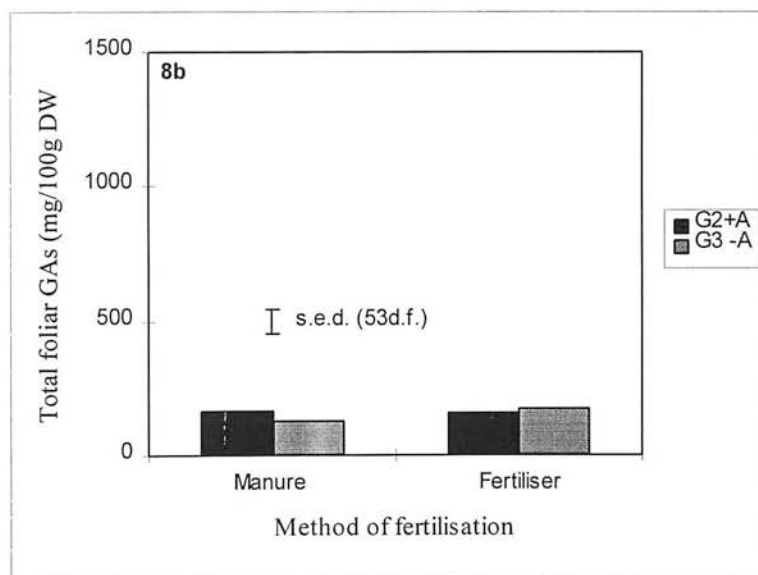
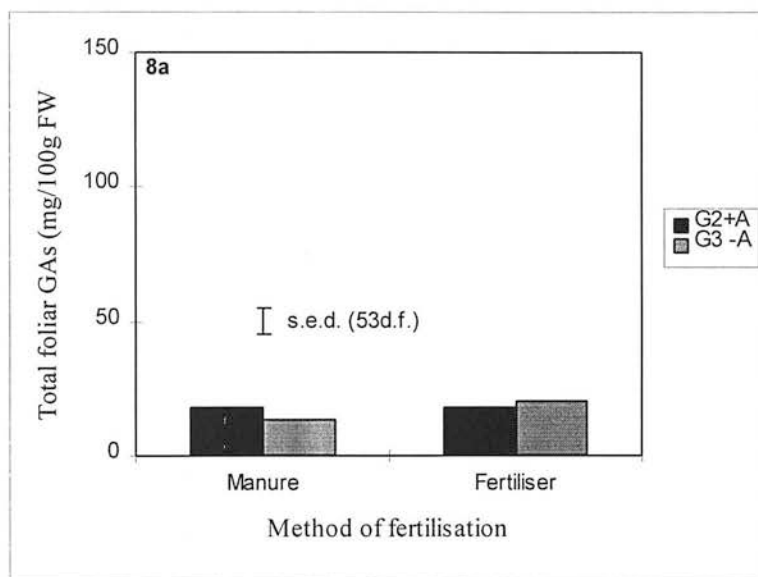
Time of harvesting did not have any significant ($P>0.05$) effect on glycoalkaloid production. Plants harvested 20, 40 and 60 days after the starting date of the experiment (G1, G3 and G5 treatments respectively) produced almost the same amounts of glycoalkaloids.

Similar results were produced by potato plants grown with fertiliser (Figures 5.7a & 5.7b). Aphid-infested plants (G2 and G4 treatments) produced almost the same amounts of fresh or dry weight glycoalkaloids as the non-infested plants (G3 and G5 treatments). The amounts of glycoalkaloids produced by G3 and G5 treatments were reduced compared to those produced by G1 treatment; however, this reduction was not significant ($P>0.05$).



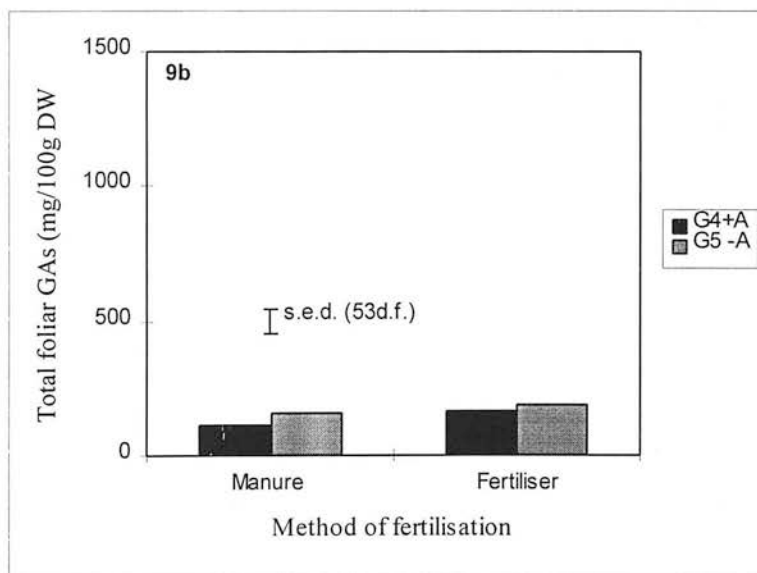
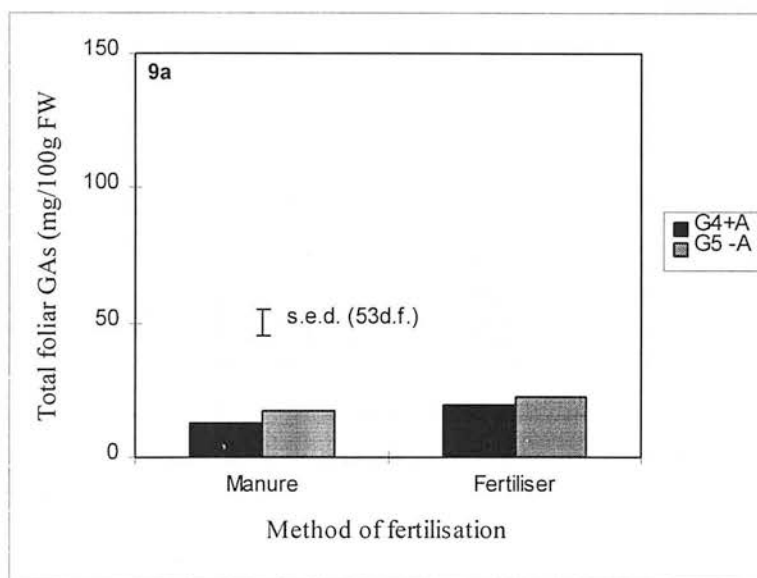
Figures 5.7a & 5.7b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids (GAs) produced by fertiliser grown, aphid-infested (I. - G2 & G4) and non-infested (N.I. - G1, G3 & G5) potato plants cv. Maris Piper, harvested at three different time intervals.

Figures 5.8a and 5.8b show the effects of fertilisation method on the total fresh and dry weight glycoalkaloid production of aphid-infested and non-infested potato cv. Maris Piper plants harvested 20 days after the aphid infestation. For both manure- and fertiliser-treated plants aphid infestation did not significantly ($P>0.05$) affect the fresh or dry weight total foliar glycoalkaloid production.



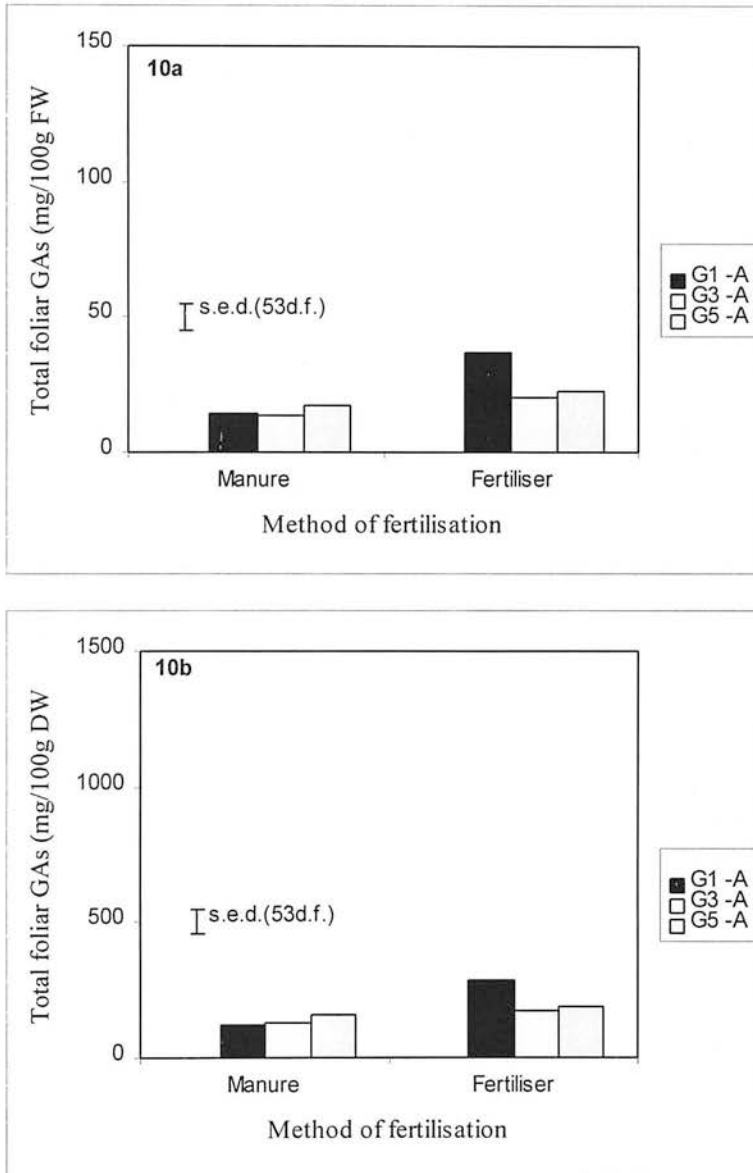
Figures 5.8a & 5.8b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids (GAs) produced by aphid-infested (G2) and non-infested (G3) potato plants cv. Maris Piper grown under manure and nitrogen fertilisation.

No significant ($P>0.05$) differences were observed in the foliar fresh or dry weight glycoalkaloids produced between manure- and fertiliser-treated plants infested by aphids (G2 treatment). In addition the difference between manure- and fertiliser-treated non aphid-infested plants (G3 treatment) was not significant ($P>0.05$). The same results were produced for aphid-infested and non-infested plants harvested at the end of the growing period (G4 and G5 treatments, Figures 5.9a & 5.9b).



Figures 5.9a & 5.9b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids (GAs) produced by aphid-infested (G4) and non-infested (G5) potato plants cv. Maris Piper grown under manure and nitrogen fertilisation.

Manure-treated plants harvested 20 days after the starting date of the experiment (G1 treatment) produced significantly ($P<0.05$) less foliar fresh or dry weight glycoalkaloids compared to the amounts produced by those plants grown with fertiliser (Figures 5.10a & 5.10b).



Figures 5.10a & 5.10b. Total content (mg/100g) of foliar fresh (FW) and dry (DW) weight glycoalkaloids (GAs) produced by non-infested potato plants cv. Maris Piper, grown under manure and nitrogen fertilisation and harvested at three different developmental stages (G1, G3 and G5).

Fertiliser treated plants harvested 20 days (G3 treatment) and 40 days later (G5 treatment) produced increased - although not significantly ($P>0.05$) - amounts of

fresh or dry weight glycoalkaloids compared to those produced by plants treated with manure.

5.3.3 Effects of fertilisation and cultivar on individual glycoalkaloid production

Table 5.3 shows the interaction effects of manure or nitrogen fertilisation x cultivar on the individual foliar glycoalkaloids produced by both potato cultivars, expressed as mg produced per 100g fresh weight. No significant difference ($P>0.05$) was observed in the amounts of solanine and chaconine produced by potato cvs. King Edward and Maris Piper.

Table 5.3. Interaction effects of fertilisation x cultivar on individual foliar glycoalkaloids (GAs) produced by potato plants cvs. King Edward (KE) and Maris Piper (MP)

Fertilisation		solanine mg/100g FW	chaconine mg/100g FW	S:C ratio
KE	Manure	31.88	60.47	35:65
KE	Fertiliser	32.05	57.62	36:64
MP	Manure	6.30	8.94	41:59
MP	Fertiliser	10.12	13.36	42:58
SED		1.55	2.69	0.018
DF			53	
P		NS	NS	NS

SED: standard error of difference between means, DF: degrees of freedom
 NS: Non significant
 solanine: total ($\alpha+\beta$) solanine (mg/100g FW), chaconine: total ($\alpha+\beta$) chaconine (mg/100g FW),
 S:C: ratio of total solanine to total chaconine, FW: Fresh weight

Type of fertilisation did not affect significantly ($P>0.05$) the α -solanine to α -chaconine ratio for either of the two potato cultivars examined. However the proportion of α -solanine in the total glycoalkaloids produced by potato cv. Maris Piper was significantly ($P<0.001$) higher than that produced by cv. King Edward for both manure- and fertiliser-treated plants.

5.3.4 Aphid performance

Table 5.4a shows the effects of fertilisation on the fecundity of the aphid *M. persicae*. No significant ($P>0.05$) difference was observed in the intrinsic rate of increase and

the total abundance of the aphids that infested manure or fertiliser-treated plants.

Table 5.4a. Effects of fertilisation on the fecundity of *Myzus persicae* adults fed on potato plants cvs. King Edward (KE) and Maris Piper (MP) grown with manure and fertiliser

Cultivar	Fertilisation	Intrinsic rate of natural increase (r_m)	Total aphid abundance (aphids/cm ²)
KE+MP	Manure	0.90	2.12
KE+MP	Fertiliser	0.40	2.40
SED		0.23	0.52
DF		11	15
P		NS	NS
SED: standard error of difference between means, DF: degrees of freedom			
NS Non significant			

The interaction effects of fertilisation x cultivar on the aphid fecundity were not significant ($P>0.05$) (Table 5.4b). The intrinsic rate of increase of aphids which was observed in the manure treated plants, was higher compared to that observed in plants treated with fertiliser. In particular this difference was significant ($P<0.01$) in potato cv. Maris Piper but not significant ($P>0.05$) in potato cv. King Edward. By contrast to the intrinsic rate of increase, the total aphid abundance was slightly ($P>0.05$) decreased in manure, compared to fertiliser treatments.

Table 5.4b. Interaction effects of fertilisation x cultivar on the fecundity of *Myzus persicae* adults fed on potato plants cvs. King Edward (KE) and Maris Piper (MP) grown with manure and fertiliser

Cultivar	Fertilisation	Intrinsic rate of natural increase (r_m)	Total aphid abundance (aphids/cm ²)
KE	Manure	0.79	1.81
KE	Fertiliser	0.44	2.17
MP	Manure	1.02	2.43
MP	Fertiliser	0.33	2.62
SED		0.2	0.74
DF		11	15
P		NS	NS
SED: standard error of difference between means, DF: degrees of freedom			
NS Non significant			

5.4 Discussion

The results obtained from chapter 4 showed that aphid infestation induced a glycoalkaloid reduction effect observed in both potato cultivars, that lasted even after the removal of the stress-inducing factor (the aphid infestation). This was attributed to the elimination of soluble sugars present in the phloem and to a decrease of the plant's photosynthetic rate. However potted field plants failed to exhibit the same aphid infestation-induced decrease in glycoalkaloid content. In addition the results from the field experiment showed that in terms of foliar glycoalkaloid production the two cultivars reacted in a different way during aphid infestation.

Aphid infestation induced an increase in the amounts of glycoalkaloids produced by aphid-infested compared to non-infested potato plants cv. King Edward. This was observed in both manure and fertiliser treated potato plants. In addition no after-effects of aphid infestation were observed in G4 and G5 treatments. By contrast, no significant difference ($P>0.05$) in the total amounts of glycoalkaloids was observed between aphid-infested and non-infested plants harvested 20 or 40 days after aphid infestation in potato cv. Maris Piper.

It has been reported (Tallamy & Raupp, 1991) that when damage is caused by arthropod herbivore feeding or by mechanical injury, leaves distant from the site of injury often accumulate compounds toxic or distasteful to arthropod herbivores and are often more resistant to feeding by these arthropod herbivores. It is possible that systemic 'induced resistance' to arthropod herbivores may have occurred. According to Hammerschmidt (1993), induced resistance to insects requires the generation, the systemic transmission, the reception and the transduction of a signal or signals. The results of the field experiment suggest this possibility at least for the potato cv. King Edward.

The presence of aphid predators and parasitoids combined with the average low summer temperatures did not allow the expected dense colonisation of potato plants

by the aphid *M. persicae*. The discrepancy observed between the results obtained from the experiments in this and the previous chapter could possibly be explained by this fact. Hence no decrease of the foliar glycoalkaloid production due to the aphid infestation was observed in the aphid-infested plants for both potato cultivars. The plant growth room and green house environments are different from the environment in the field, in factors known to influence the leaf glycoalkaloid content and more particularly soil nitrogen. By contrast to tuber glycoalkaloids, no information exists on how other factors such as soil potassium, near-UV radiation, temperature and relative humidity are affecting the glycoalkaloid concentration in potato foliage. The generation of a systemic signal produced by a low number of aphids on the protected (from aphid predators) lower potato leaves could explain the increase of foliar glycoalkaloids observed in potato cv. King Edward. This signal possibly induced the increase of the foliar glycoalkaloids produced in both manure- and fertiliser-treated plants of potato cv. King Edward. In addition removal of the aphids from the plants brought glycoalkaloids back to the levels prior to aphid infestation. However it seems that for some reason such a signal did not occur in potato cv. Maris Piper.

The manure application resulted in elevated aphid intrinsic rates of increase in both potato cultivars. By contrast the aphid abundance was slightly reduced in the manure treated plants. Using the intrinsic rate of increase and the aphid abundance as a measure of the aphid reproductive performance in the plants may introduce a big difference in the evaluation of the results because both are not similarly dependent on the physiological condition of the host plant. In some cases aphids that had been parasitized, were observed during the measurement of the aphid abundance, hence the presence of aphid parasitoids could not be excluded from being a factor that possibly increased variation. The measurements for the intrinsic rate of increase were taken in clip cages protected from aphid parasitoids, while the aphid abundance was estimated in a less protected environment (the nylon mesh pocket).

In addition the difference observed between these two fecundity parameters, could possibly be explained by the fact that the measurements of the aphid intrinsic rate of

increase and those of aphid abundance were taken in different parts of potato plants (second true leaf, upper leaves and first true leaf respectively). It seems that the younger leaves of manure treated plants consist a better environment for aphid development compared to younger leaves of fertiliser treated plants. This could possibly be attributed either to higher nutritive quality of the phloem sap in young leaves of manure treated plants, or to the existence of antifeeding agents in lower amounts. However the latter seems quite unlikely, since manure treated plants produced significantly more amounts of glycoalkaloids compared to fertiliser treated plants. It follows that the nutritive quality of phloem sap is possibly better in younger and by contrary poorer in older leaves of manure treated plants, possibly because of elevated or reduced amounts of soluble nitrogen respectively. Stockdale *et al.* (1992), reported increased aphid numbers early in the season on potatoes and supported that manure applied in increasing quantities to plants, may lead to increased aphid problems. Even at a constant level of soluble nitrogen compounds in the leaves the concentration of certain amino acids in the phloem sap may change considerably (Harrewijn, 1970). This additionally may explain the more or less confusing results. Differences in microclimate between faster and slower developed plants may also be mentioned as a possible cause of variation in reproduction of aphids. Answers to these questions could possibly be gained, if analyses for soluble nitrogen (amino acids, nitrate nitrogen) and glycoalkaloids, could be performed separately in individual potato leaves.

In general manure treated potato cv King Edward plants, harvested early in G1 treatment produced decreased amounts of glycoalkaloids compared to fertiliser treated plants (Fig. 5.5a & 5.5b). This was observed as well for potato cv. Maris Piper (Fig. 5.10a & 5.10b). The amounts of green matter harvested in G1 treatment were almost the same for both manure and fertiliser treatments for each potato cultivar (Table 5.2c). Hence the increase of glycoalkaloid production in G1 treatment observed in fertiliser grown plants could not be attributed to differences in green matter from manure and fertiliser treatments simulating a dilution effect. These results are in agreement with those obtained by Hellenas & Branzell (1995),

for potato tubers and a possible explanation could be attributed to the different rates of nitrogen release and plant uptake between plants treated with manure and those treated with fertiliser.

In conclusion, the results suggest that low densities of aphid infestations could induce a temporary increase of potato foliar glycoalkaloids. These results support the idea of a natural defence system existing in the potato plant. The results also suggest that because no differences in glycoalkaloid levels between manure and fertiliser treated plants were observed, the source of available nitrogen does not play any role in glycoalkaloid synthesis.

Chapter 6

General discussion, conclusions and future prospects

6.1 General Discussion

Glycoalkaloids are generally regarded to be a promising source of resistance against potato crop pests. The most desirable potato plant would ideally have a low glycoalkaloid content in the tubers to reduce the potential health hazard to humans and high levels in the leaves and stems to provide protection against insects and pathogens. The experiments of the second chapter showed that ingestion of an artificial diet containing 40 mg/100ml or greater of potato glycoalkaloids by *M. persicae* induced a suppression in the life parameters of the aphid such as fecundity, longevity, mean relative growth rate and diet uptake. According to Percival & Dixon (1997), although the antifeedant properties of glycoalkaloids on humans and animals are well documented, the biochemical basis of their effects has not been sufficiently explained. In addition it is still unknown in which way the aphid physiology is affected by these toxins. Their main mode of action is considered to be the inhibition of cholinesterase activity (Roddick, 1989; Bushway *et al.*, 1987) which results in the blocking of nerve transmission. Additionally they are regarded (Fewell *et al.*, 1994; Roddick *et al.*, 1988; Roddick & Rijkenberg, 1986) as acting as cell membrane disruptive and destabilising agents. Since Roddick (1979), concluded that the steroidal potato glycoalkaloids are able to complex with free sterols (cholesterol, sitosterol, stigmasterol etc.) *in vitro*, it could be assumed that glycoalkaloids could interfere somehow with the sterol biochemistry of the aphid when ingested from an artificial diet.

Apart from the suppressed performance observed in glycoalkaloid concentrations similar to those detected in the potato foliage (equivalent to 40-160 mg GAs /100ml diet), the results indicated a short term stimulatory effect on aphids reared at low concentrations (10-20 mg GAs /100ml diet). Similar results were obtained from the study of Güntner *et al.* (1997), who found that a low concentration of α -chaconine in a meridic diet induced feeding stimulation by the aphid *M. euphorbiae*. Additionally they suggested that this aphid is well adapted to potato glycoalkaloids.

It is generally accepted that insects do not have the capacity to synthesize sterols but do need a dietary supply of sterol, usually cholesterol for normal development (Srivastava, 1987; Clayton, 1964). As has been concluded for *A. pisum*, aphids are not capable of *de novo* sterol biosynthesis but depend on an endogenous source – perhaps their symbiotic bacteria- to synthesize them. In particular (Griffiths & Beck, 1977a, 1977b, Houk *et al.*, 1976) concluded that sterol is synthesized and exported by symbionts in *A. pisum*. This was also implied by the rearing of *A. pisum* for 28 generations (Akey & Beck, 1972) and that of *M. persicae* in permanent culture (Dadd & Mittler, 1966) without an exogenous source of sterols. These bacteria located in the mycetocyte cells in the haemocoel, are believed to complement the aphid diet by the provision of B vitamins (Ehrhardt, 1968), essential amino acids (Houk & Griffiths, 1980), and possibly sterols (Douglas, 1988; Douglas, 1990). According to Harrewijn (1978), sterols are not needed in an artificial diet, because in a healthy aphid the symbionts are able to produce them at an adequate level (Ehrhardt, 1968). However additional evidence suggests that the symbiotic bacteria in some aphids do not synthesize cholesterol. According to Forrest & Knights (1972), *M. persicae* is not relying completely on its symbionts for sterol synthesis, as many of its host plants probably can provide this aphid with sterols. Campbell & Nes (1983), determined that *S. graminum* is not capable of incorporating either radio labelled ^{14}C -acetate or ^3H -mevalonic acid into cholesterol. In addition Campbell & Nes (1983), were able to demonstrate the bioconversion of ingested phytosterols into cholesterol, which is regarded as the precursor of solanidine during glycoalkaloid biosynthesis in Solanaceae (Jadhav & Salunkhe, 1975). Srivastava (1987), concluded that the sterols in the phloem sap of many plants exist in such quantities that they are sufficient to meet the minute needs of aphids.

The aphid, by monitoring the chemistry of the substrate during stylet penetration from epidermis to the sieve element of a potato leaf, is likely to be detecting traces of glycoalkaloids. The alternative hypothesis-that aphids never contact these metabolites- cannot be eliminated, but according to Güntner *et al.* (1997), Harrewijn (1990), Niemeyer, (1990), van Emden (1972), this possibility seems remote. In

comparative studies between two *M. persicae* biotypes reared on radish and *Nasonovia ribisnigri* (Mosley) reared on lettuce, Harrewijn (1990), concluded that the resistance mechanism is not the same for these two aphid species and is probably based upon both antixenosis and antibiosis. *N. ribisnigri* encounters resistance from both the plant surface and the phloem tissue, while *M. persicae* encounters resistance in the mesophyll and during phloem feeding. In addition there is an indication that glycoalkaloids are not present in the potato phloem (Roddick, 1982). Further phloem sap and aphid honeydew analyses, performed in aphids feeding on potato foliage, are required to examine if these insects are absorbing glycoalkaloids through feeding on the potato. Furthermore, comparative studies involving the analysis of honeydew samples derived from aphids feeding on glycoalkaloid-containing artificial diets and those derived from foliage feeding aphids, could shed light on the question whether aphids come into contact with glycoalkaloids during feeding on the potato.

The steroidal structure of glycoalkaloids, linked to the aphids' necessity for sterols, could suggest an essential role for these metabolites in aphid nutrition, possibly related to chemical stimulation. The aphids reared on the glycoalkaloid-containing and glycoalkaloid-free diets, were not lacking symbiotic bacteria; hence according to Griffiths & Beck, (1977), Griffiths & Beck (1977), and Houk *et al.* (1976), in both diets the insects were able to obtain sterols *in vivo* from their symbionts. However these symbiotic aphids performed better in the low glycoalkaloid concentrations (10-20 mg GAs /100ml diet) compared to controls (0 mg GAs /100ml diet). Douglas (1996), concluded that elimination of the symbiotic bacteria *Buchnera* from young larval aphids, resulted in a far greater reduction in the growth of the embryo fraction of the aphid than of the maternal tissues. It is not known if potato glycoalkaloids could affect either the symbiotic bacteria through interference in the sterol biosynthesis pathway, or the aphid *per se* through a mechanism not associated with its symbionts. The answer to this question could be obtained if the aphid-bacteria symbiotic system could be examined separately. The production of aposymbiotic aphids is possible by using high concentration of antibiotics in artificial diets. By contrast, although symbiotic bacteria can be isolated *en masse* from host tissue, they

have not been maintained yet in continuous culture (Houk, 1987).

As previously suggested, traces of glycoalkaloids may contribute to aphid nutrition providing the insect with essential amounts of sterols, that under natural conditions are provided by the aphids' symbiotic bacteria. This hypothesis could be further investigated by rearing aposymbiotic *M. persicae* and *M. euphorbiae*, on artificial diets containing low concentrations of glycoalkaloids. Furthermore since all aphid species are expected to require sterols for their development, it would be important to examine if a possible phagostimulant effect of glycoalkaloids exists in aphid species that typically do not infest potato; a good case could be that of *A. pisum* or *B. brassicae* which have host ranges restricted to Leguminosae and Cruciferae respectively. This could be achieved by comparing the performance of symbiotic and aposymbiotic forms of these species on artificial diets containing low concentrations of glycoalkaloids.

To summarise this part of the discussion, the adverse effects observed when glycoalkaloids were presented to *M. persicae* in artificial diets could possibly be attributed either to the toxicity that these substances may induce on the insects' feeding and reproductive physiology, or to the interference in the symbiotic relationship that *M. persicae* has developed with its endosymbiont bacteria or both. Since glycoalkaloids proved to have toxic or antifeeding effects on the aphid *M. persicae*, it would be expected that these chemicals constitute a repellent stimulus or a source of toxicity. This may in principle be manipulated by plant breeders to generate plants with enhanced barriers to aphid colonisation. However the reduced performance of *M. persicae* observed in the glycoalkaloid-containing artificial diets does not imply that a complete resistance mechanism has been discovered. Additional experimentation, involving electrical recording of stylet penetration activities of *M. persicae* on artificial diets containing glycoalkaloids, is essential to examine whether declined longevity and fecundity, observed in the higher (80-160 mg GAs/100ml diet) glycoalkaloid treatments, were due to toxic effects of ingested glycoalkaloids, or feeding deterrence of the diets with subsequent starvation.

According to Harrewijn (1990), the evaluation of the resistance factors that operate once the aphids have inserted their stylets into a plant, is a complicated procedure. The only reliable way to know where the resistance factor is located, is by electrical recording of probing activities combined with high resolution microscopy, electron microscopy and stylet cutting to obtain phloem sap. It has been suggested by Harrewijn (1990), that for the characterisation of resistance mechanisms, comparative studies using artificial diets should be performed on more than one aphid species. It follows that parallel studies using the same type of artificial diets (either holidic or meridic), containing equal amounts of glycoalkaloids should be accomplished for *M. persicae*, *M. euphorbiae* and *A. solani*. The results from these experiments should be interpreted taking into account the individual feeding habits and host searching behaviour of each species. For example, attention should be paid to the fact that *M. persicae* is considered to be less specific in feeding preferences compared to *M. euphorbiae*, which is even more restless and moves more rapidly from plant to plant (Jones & Jones, 1984).

Although all glycoalkaloids destabilise cholesterol-containing biomembranes, α -chaconine is regarded to be more effective than α -solanine (Roddick & Rijnbergen, 1986; Keukens *et al.*, 1996; Roddick *et al.*, 1990; Steel & Drysdail, 1988; Roddick & Rijnbergen, 1987). Alpha-solanine is able to complex with sterols *in vitro*, but cannot produce lytic effects except at pH values of 8.0 and above (Roddick & Rijnbergen, 1986). In addition it has been reported that α -chaconine has stronger antifungal action compared to α -solanine (Fewell *et al.*, 1994; Fewell & Roddick, 1993). Alpha-chaconine proved to be more toxic against embryos of the frog *Xenopus* (Friedman *et al.*, 1992; Friedman *et al.*, 1991). The importance of the intact carbohydrate moiety was demonstrated by the inability of β -chaconine (α -chaconine with one sugar monosaccharide removed) to complex with sterols or disrupt liposomes (Roddick & Rijnbergen, 1986). Rayburn *et al.* (1994), concluded that the biological activity of glycoalkaloids is influenced by the chemical structure of the carbohydrate (i.e., galactose, glucose, rhamnose); the number of carbohydrate groups making up the side chain attached to the 3-OH position of the aglycon solanidine;

and the stereochemical orientation of the chaconine diglycosides. In general the toxicity of these compounds to developing *Xenopus* embryos generally decreased following removal of the carbohydrates from the triglycosides (Rayburn *et al.*, 1994). These results suggest that the toxic effects of glycoalkaloids are strongly dependent on the structure of both their aglycone and their oligosaccharide moieties. The normal survival of *M. persicae* nymphs reported by Schoonhoven & Derksen-Koppers (1976) may be due to the exclusion of α -chaconine from the artificial diets presented to the aphids. This is additionally supported by the results of Güntner *et al.* (1997) who suggested that α -solanine increased the reproduction rate of *M. euphorbiae*, while α -chaconine produced a strong decrease.

The 40:60 α -solanine to α -chaconine ratio applied in the artificial diet experiments of chapter 2, reflects the average proportion reported by analyses performed on tissue samples derived from potato tubers. The results from the foliar glycoalkaloid analyses of potato cvs. King Edward and Maris Piper performed in chapter 3, proved that this ratio fluctuates, depending on nitrogen content of nutrient solutions, but mostly with potato cultivar. In particular, nitrogen fertilisation increased the amounts of α -solanine produced in both cultivars, while cv. King Edward produced more α -chaconine compared to that produced by cv. Maris Piper. The decreased total glycoalkaloid content in combination with the higher –but less toxic- α -solanine content detected in potato cv. Maris Piper, may contribute to the slightly better performance of the aphid observed in this cultivar. In addition, the appearance of the secondary peaks in potato cv. Maris Piper, which were attributed to the –possibly less toxic (Roddick & Rijnenberg, 1986)- β - forms of solanine and chaconine, could raise further implications for the role that glycoalkaloids play in potato defensive mechanisms. Studies using artificial diets of different α -solanine to α -chaconine profiles, could further examine the individual importance of each of these secondary potato metabolites for aphid species that infest potato.

It is generally accepted that elevated amounts of nitrogen applied to crops induce elevated infestations by pests. Early studies reported that elevated nitrogen dosages

increase aphid numbers by increasing the soluble nitrogen levels in the foliage (van Emden *et al.*, 1969; Wooldridge & Harrison, 1968; van Emden, 1966). Evidence from the existing literature suggests that the population development of *M. persicae* may be associated with foliar concentrations of amino-nitrogen compounds which are mobilised during leaf senescence. Harrewijn (1978), suggested that changes in the content and proportions of nutrients in the phloem sap could have a profound effect on the rate of growth, fecundity and polymorphism of *Myzus persicae*. However the literature concerning the relations between the performance of *M. persicae* and soluble nitrogen compounds in potato foliage is sometimes confusing. Van Emden & Bashford (1969), showed that fecundity of *M. persicae* was positively correlated with concentrations of soluble nitrogen within leaves of the same age but not between leaves of different ages in Brussels sprout. In addition, Harrewijn (1970), concluded that the levels of total and soluble nitrogen in potato leaves and the reproduction rate of *M. persicae* were positively correlated with the amounts of nitrogen applied to potato plants through nutrient solutions. Jansson & Smilowitz (1986), showed that population parameters of *M. persicae* increase with an increase in soluble nitrogen or free amino acids in potato plants fertilised with different levels of nitrogen fertiliser. This study also showed that the population growth rate of *M. persicae* is influenced by slight changes in the concentrations and proportions of amino-N compounds and nitrate-N in leaves. Populations of *M. persicae* grew more rapidly on lower leaves which had higher concentrations of nitrate N than upper leaves, which were lower in nitrate N.

Although nitrogen fertilisation can alter the mechanisms involved in host plant resistance, it is uncertain whether these effects are due to changes in the nutrient content or due to changes in the production of secondary metabolites. Increased rates of nitrogen fertilisers make the plant more succulent by increasing tissue softness and by increasing water content (Dale, 1988). All leaves appear to provide similar physical environments to less mobile insects such as aphids, planthoppers, and leafhoppers; but they provide different quantities of resources because the photosynthate production is proportional to leaf area (Dale, 1988). The results

obtained in chapter 3 suggested that elevated nitrogen fertilisation applied to potato plants induced a gradual reduction of foliar glycoalkaloid production; this was attributed to a dilution effect of glycoalkaloids in an increasing amount of green matter. The highest glycoalkaloid contents were observed in the nitrogen deficient plants. Since glycoalkaloids are regarded as factors contributing to the resistance against potato pests, it can be positively assumed that elevated nitrogen fertilisation affects negatively that part of the potato plants' defence mechanism which is related to the secondary metabolism of potato. This suggests that elevated nitrogen fertilisation could result in better aphid performance, either due to the enrichment of the sap with soluble nitrogen forms (amino acids or nitrate nitrogen), or due to the induced reduction of the total glycoalkaloid content, or both. However the elevated glycoalkaloid content typically detected in potato tops and sprouts, does not prevent aphids from feeding on them.

Secondary compounds are so called because they do not have an essential role in the primary life processes of plant cells and because they are not present in all plants (Rhodes, 1985). It has been suggested that although secondary compounds are not considered as nutritious substances, they interact with nutrients present in plant tissues (Reese & Schmidt, 1986). Reese (1979), has stated that interactions between some defensive chemicals and nutrients can make nutrients unavailable even if they are present in large quantities. There is increasing evidence that secondary metabolic products exist in a dynamic balance and are involved in cycles that include primary compounds such as sugars and amino acids (Seigler & Price, 1976). Glycoalkaloid synthesis is controlled by a multi-enzymic system of plant secondary physiology and is possibly dependent on the amounts of carbohydrates available for glycosylation. Alteration of the nitrogen nutrition of a plant is affecting the concentrations of the soluble nitrogen forms (nitrate nitrogen, amino acids), as well as the concentrations of soluble sugars.

Secondary plant metabolism plays a crucial role in plant-herbivore interactions. A plant may lower its nutritive quality for herbivores by using secondary compounds,

morphological characters and/or having a lowered nutrient content. If these methods decrease the amount of resources lost through herbivory, then they act as antiherbivore defences. However if herbivores compensate for the lowered nutrient availability by increasing their intake rates or by prolonging their feeding periods, then this may render the defence useless (Augner, 1995). Aphids, during feeding on a plant cause direct physical damage and in addition induce biochemical and physiological changes within the plant, including plasmolysis of the cells. Plants respond to aphid infestation very rapidly, even in parts away from the site where the aphids are feeding (Harrewijn, 1978). Plastids can degenerate or decrease in size and number, and chloroplasts become colourless (Pollard, 1973). Dense aphid colonisation of potato plants caused a dramatic reduction of total and individual glycoalkaloid synthesis in the potato foliage as reported in chapter 4. It was shown in most cases that GA reduction was permanent: even after infestations were removed from potato plants, the GA production of infested and control plants can continue to diverge over time. This reduction was attributed to the depletion of carbohydrates essential for glycoalkaloid synthesis which derived either from their ingestion by the aphids, or from the suppression of photosynthetic rates in the aphid-infested foliage, or both. An additional reason could be the depressed production of solanidine due to the reduction of undamaged plastids that occurred in the cells of potato foliage.

However such reduction in glycoalkaloid content was not observed in the experiment that took place in the field. The glycoalkaloid production of the aphid-infested plants was increased, at least for potato cv. King Edward. A possible explanation of the discrepancy observed between the plant growth room and field experiments with respect to insect-induced stress could be related to the effects that aphid infestation have on the plants' nutritional physiology which further affects the concentrations of defence chemicals. Harrewijn (1976), showed that the feeding of *M. persicae* at low densities increases the growth of potato plants growing in media containing relatively low concentrations of phosphorous by stimulating the plants' nutrient uptake. Further investigation involving measurements in the carbohydrate and soluble

nitrogen status in potato plants could explain the decrease or increase of glycoalkaloid content observed in aphid infested plants grown in the plant growth room and in the field experiments respectively. Since the methods measuring the aphid performance in these experiments were taking into account the plants' reaction following a dense aphid colonisation, further studies could shed light on how glycoalkaloid concentrations existing in potato leaves may affect the individual aphid performance or, vice versa, i.e. how glycoalkaloid concentrations are affected by very low aphid densities. Possibly glycoalkaloids during the initial stages of infestation serve in the potato plant foliage to induce a short term antixenosis effect; however, aphids are able to suppress further glycoalkaloid formation by removing sugars and soluble nitrogen from the leaves when densely colonising the plant.

Hlywka *et al.* (1994), reported higher glycoalkaloid concentrations in tubers of Colorado potato beetle-damaged plants which were not associated with tuber yield or size characteristics; they concluded that a food crop not protected from common pests may produce elevated levels of natural toxins affecting the degree of food safety. However the results from chapter 4 suggest that glycoalkaloid synthesis should not be examined separately from of primary physiological functions such as carbohydrate formation, photosynthetic rates, or nitrogen mobilisation and nutrition. There is increasing evidence that secondary metabolic products exist in a dynamic balance and are involved in cycles that include primary compounds such as sugars and amino acids (Seigler & Price, 1976; Reese & Schmidt, 1986). Even if the mechanism for induction of glycoalkaloid synthesis has yet to be determined (Bergenstrahle *et al.*, 1992a, 1992b), it seems that the glycoalkaloid synthesis response is multigenic (Sinden *et al.*, 1984). Hlywka *et al.* (1994), suggested that if the increased synthesis of glycoalkaloids in the tubers is a result of a direct pest interaction with the foliage, there must exist some signal-response mechanism. This is further supported by the fact that glycoalkaloids are not translocated within plants (Roddick, 1982). An increasing number of examples suggests that there is a secondary metabolite stress response in plants to insect infestation. Accumulation of nicotine has been associated with induced resistance to arthropod herbivores in

tobacco. Feeding or mechanical injury of tobacco leaves resulted in the accumulation of nicotine (Baldwin, 1988a, 1988b, 1988c). Wool & Hales (1996), observed that cotton seedlings that survived heavy infestation by the cotton aphid *A. gossypii* were resistant to cotton aphid recolonisation. Furthermore these authors suggested that the damage caused by the first infestation-and not the production of some excess metabolites- was the reason for the lower success of the aphid on previously infested plants. It has been found that aphid feeding can provoke an increase in hydroxamic acid levels in some wheat cultivars; this result opens the possibility of diminishing aphid-provoked viral infections in wheat (Niemeyer, 1988). Studies should be addressed to examine the glycoalkaloid synthesis in sites distant from the point of the initial aphid infestation.

It has been recognised that the biochemical changes that occur in the host plant during aphid infestation play a key role in regulating the interactions not only between the plant and the aphid and but also between the plant and the natural enemies of the aphid. In addition to direct effects of herbivores, defensive chemicals have been shown to play a role in the effectiveness of natural enemies (Barbosa & Saunders, 1985; Schultz, 1983). Mittler, (1988), concluded that the most crucial area of aphid biology which demands study from the basic as well as the applied points of view, is the effect that chemicals from the surface and wax layers of plants have on the probing and feeding behaviour of aphids, on host and non-host plants, on plants at different stages of development, as well as on susceptible and resistant varieties. Probing behaviour is presumed to provide the aphid with the information on the physical and chemical properties of the substrate. According to van Emden *et al.* (1969), the stage of probing has huge potential for the manipulation of the host plant in the control of aphids, because the acceptability of a particular host is often determined at this stage. As far as chemicals are involved in the selection process both secondary substances and nutrients are important for acceptance or rejection of a plant as a host (Klingauf, 1987).

An aphid prior to phloem feeding has to overcome the defensive systems of the plant,

some of which are involved with host plant recognition by the aphid. In addition to the secondary plant metabolites, sticky hairs or glandular pubescence and the release of E- β -farnesene which is an aphid alarm pheromone contained in *S. berthaultii* trichomes, are reported to contribute substantially to plant resistance during the attack phase (Ave *et al.*, 1987; Gibson & Pickett, 1983; Tingey & Sinden, 1982; Tingey & Laubengayer, 1981; Gibson, 1971). It has been reported that the concentration of E- β -farnesene in the foliage remains at levels sufficient to result in avoidance by the aphids (Gibson & Pickett, 1983). Hartmann (1991) suggested that a chemical defensive system such as alkaloids to be effective, has to be localised at the site to be protected; the alkaloid concentration has to be sufficient to account for chemical defence. The peach potato aphid *M. persicae* settles on tobacco leaves in large colonies, although nicotine as a natural component of tobacco leaves is very toxic to the aphid and may be used as an effective aphicide. Whereas nicotine can accumulate in leaf cells of some *Nicotiana* species, the phloem sap contains only traces of the alkaloid. In addition it must be taken into account that although the availability of large numbers of individuals of similar genetic constitution make aphid clones valuable for many experimental purposes, the local wild populations and laboratory cultures of an aphid are likely to contain much less of the genetic range of a species than where sexually reproducing insect populations are concerned. Van Emden *et al.* (1969), concluded that generalisations from laboratory or local populations are therefore dangerous especially when the species, like *M. persicae* is extremely widely distributed in many different tropical and temperate habitats. It follows that for *Solanum* sp. the toxicity of glycoalkaloids alone observed in artificial diet experiments does not establish their role in plants' chemical defence against *M. persicae*.

According to Hartmann (1991), the understanding of an assigned defensive function of a certain alkaloid, requires knowledge of its biosynthesis and metabolic behaviour, as well as the strategies by which the defensive chemical is presented and the mechanisms realising these strategies. The role of glycoalkaloids in plant resistance to insects is of practical interest because it has been shown that these compounds are

heritable and depend on the parental glycoalkaloid composition (Van Gelder *et al.*, 1988; Gregory, 1984). Efforts should be directed towards understanding their mode of action on the recipient organism as well as their biosynthesis. In addition, efforts should be devoted to define their precise location in the plant, with particular focus on the chemical nature of phloem sap, the nature and quantity of materials ingested by aphids and their fate in the aphid. Testing the role of the potato glycoalkaloids in resistance of potato hybrids by breeding with wild *Solanum* species may contribute to increased potato resistance to insects. This could lead to a more rational approach towards crop improvement, potentially permitting the attainment of more stable resistance by the use of genetic engineering techniques. Hence an understanding of the genes involved in the accumulation (biosynthesis vs. degradation) of glycoalkaloids is essential if a bio-engineering approach is to be undertaken. In addition the effects of a potential increase in glycoalkaloid levels in potato on other potential consumers, should be considered.

6.2 Summary of major findings

1. This project demonstrated the toxic effects of the potato glycoalkaloids α -solanine and α -chaconine, on the life parameters of the *M. persicae* adults and nymphs, when presented in artificial diets in concentrations and proportions similar to those typically detected in the potato foliage.
2. Low glycoalkaloid concentrations had a short term stimulatory effect on *M. persicae* nymphs and adults, expressed as elevated fecundity, relative growth rates and diet uptake.
3. The slightly acidic pH of the diet did not reduce the aphid performance either in the adult or in the nymph experiment.
4. Elevated nitrogen fertilisation induced a gradual reduction of foliar glycoalkaloid content (mg / 100g fresh or dry weight) in both potato cultivars, possibly due to a dilution effect associated to a higher yield of green matter.
5. The total glycoalkaloid yield (mg / plant) produced in potato foliage was increased with elevated nitrogen fertilisation in both potato cultivars, due to

- the increased amounts of green matter produced.
6. Elevated nitrogen fertilisation increased the amounts of α -solanine produced in both potato cvs King Edward and Maris Piper. Potato cv. Maris Piper produced less total foliar glycoalkaloids compared to potato cv. King Edward.
 7. The proportion of α -solanine to α -chaconine was decreased in potato cv. King Edward which produced elevated amounts of α -chaconine compared to those produced by potato cv. Maris Piper.
 8. Pesticide application produced confusing results for potato cv. King Edward and had no effect on total foliar glycoalkaloid production for potato cv. Maris Piper. In particular (Chapter 3 exp.1), the glycoalkaloids produced in cv. King Edward in the control treatment (no pesticide - P_0) were significantly higher ($P < 0.01$) compared to those produced in the P_1 and P_2 treatments. By contrast in the second experiment of the same chapter, significantly more glycoalkaloids ($P < 0.05$) were produced in P_1 treatments compared to control, while no differences were observed between control and the highest (P_2) pesticide treatment.
 9. Slight modification in the acetonitrile/water proportion made possible the detection of secondary peaks in the HPLC chromatograms which were attributed to β - forms of solanine and chaconine.
 10. Dense aphid colonisation of potato plants induced a reduction in the amounts of glycoalkaloids produced in the foliage. This was due to a possible concurrent depletion in the amounts of soluble carbohydrates, derived either from the lower photosynthetic rates, or from their removal by the aphids through feeding in the phloem.
 11. In the field experiment aphid infestation induced an increase in the amounts of glycoalkaloids produced by potato plants cv. King Edward grown with manure or fertiliser. However this was not observed in potato cv. Maris Piper plants.
 12. Manure application resulted in elevated aphid intrinsic rates of increase in both potato cultivars and increased amounts of foliar glycoalkaloids in aphid infested and non-infested plants.

The toxic effects of potato glycoalkaloids on the aphid when presented in artificial diets in high concentrations establish their potential as naturally occurring pesticides and give rise to the possibility glycoalkaloids being an additional barrier against aphid infestation. An increase in the foliar glycoalkaloid concentrations could suggest an eventual reduction in the use of synthetic pesticides. In this case breeding for high glycoalkaloid levels in the foliage and low levels in the tubers should be the plant protection objective. Artificial diet results should be interpreted with caution because they are useful only as an indication regarding the toxic effects of these substances when presented in pure form. The reduction in the amounts of foliar glycoalkaloids produced due to excessive nitrogen fertilisation suggests a more rational approach in the use of nitrogen resources due to their collateral effects on the potato crop defence system. High nitrogen fertilisation rates applied to conventional potato crops, could result in an increase of their susceptibility to pest infestation, due to a concurrent reduction of natural defensive toxins. Hence nitrogen fertilisation could be an indirect way of pest management because it can alter the concentrations of natural occurring toxins. It is safe to conclude that the glycoalkaloid levels in potato foliage could be altered by adjusting the levels of applied nitrogen. This should be taken into account when planning nutrition and crop protection schedules in organic or conventional farming. Suggesting that nitrogen availability affects aphid infestation through secondary plant metabolism, a correlation between nitrogen fertilisation and foliar glycoalkaloids as well as between foliar glycoalkaloids and aphid performance should be established. Aphid performance depends on both phloem sap quality and foliar glycoalkaloid concentrations. A negative effect of nitrogen fertilisation on glycoalkaloid synthesis has been found. Although a negative correlation between aphids feeding *in situ* and foliar glycoalkaloids has been established, the causal effect between foliar glycoalkaloids and aphid infestation development should be examined. However the increase of the foliar glycoalkaloid content observed due to aphid infestation in the field, is supporting the idea of the promotion of a natural defence system of potato crop which could prevent further pest attacks.

6.3 Recommendations for future work

1. Analyses of aphid honeydew deriving from potato-feeding aphids and potato phloem sap samples, could investigate the presence of glycoalkaloids in the aphid diet *in situ*.
2. Further work investigating the actual concentration of glycoalkaloids in the mesophyll cells is required for an estimation of the actual amounts imbibed by the aphid before the aphid stylet reaches the final feeding site.
3. Rearing of aposymbiotic and symbiotic *M. persicae* and *M. euphorbiae* on low glycoalkaloid artificial diets could provide useful information regarding the role of glycoalkaloids as a source of sterols. This could be additionally investigated by the rearing of aphid species which typically do not infest potato on low glycoalkaloid artificial diets.
4. Electrical recording of stylet penetration activities on high glycoalkaloid artificial diets could examine if the declined performance observed is due to toxic effects of these substances or due to feeding deterrence and starvation.
5. Comparative studies of rearing *M. persicae*, *M. euphorbiae* and *A. solani* on artificial diets containing different concentrations and ratios of α -solanine to α -chaconine could provide further information about the general defensive profile of potato against aphids.
6. Studies measuring the concentrations of soluble sugars as well as the soluble nitrogen forms (nitrate nitrogen, amino acids) in leaf tissues are essential to indicate any correlation with glycoalkaloid levels.
7. Additional research possibly using radio labelling techniques could investigate the movement of nitrogen in the enzymic system involved in glycoalkaloid synthesis.
8. Since β - forms of solanine and chaconine are considered to be less toxic than α - forms, a better separation of peaks detected through an improved HPLC analytical technique could lead to useful conclusions regarding the chemical defensive profiles of different potato cultivars.
9. Measurements of the carbohydrate and soluble nitrogen status (amino acids

and nitrate nitrogen) in potato foliage could explain the decrease of glycoalkaloid content observed in the plants suffering dense aphid colonisation.

10. Correlation of aphid fecundity (using the method of the intrinsic rate of increase) on plants that are not densely colonised with glycoalkaloid and soluble nitrogen content could contribute to the understanding of aphid-glycoalkaloid relationships *in situ*.
11. Examination of the glycoalkaloid profile in separated individual leaves distant from the site of initial aphid infestation could investigate a potential short term stress reaction possibly indicated by an increase in the amounts of glycoalkaloids produced.
12. Further studies could investigate how aphid infestation affects the amounts of tuber glycoalkaloids and the mechanisms involved e.g. by altering the carbohydrate and soluble nitrogen content required for tuber formation.

Bibliography

- Adams, J.B. & van Emden, H.F.** 1972. The biological properties of aphids and their host plant relationships. In: van Emden HF (ed.), *Aphid Technology*. Academic Press, London, pp. 47-104.
- Agerburg, L.S., Schick, R., Schmidt, M. and Sengbusch, R.V.** 1933. Die Bestimmung des Solaningehaltes von Pflanzen mit Hilfe von *Cladosporium fulvum*. *Zuchter* **5**: 272-280.
- Ahmed, S.S. & Müller, K.** 1978. Effect of wound damages on the glycoalkaloid content in potato tubers and chips. *Lebensmittel Wissenschaft und Technologie* **11**: 144-146.
- Ahmed, S.S. & Müller, K.** 1979. Seasonal changes and the effect of nitrogen-and potash- fertilization on the solanine and the a-chaconine content in various parts of the potato plant. *Zeitschrift für Pflanzenernährung und Bodenkunde* **142**: 275-279.
- Akey, D.H. & Beck, S.D.** 1972. Nutrition of the pea aphid, *Acyrtosiphon pisum*: requirements for trace metals, sulphur and cholesterol. *Journal of Insect Physiology* **18**: 1901-1914.
- Al-Mousawi, A.H., Richardson, P.E. and Burton, R.L.** 1983. Ultrastructural studies of green bug (Hemiptera: Aphididae) feeding damage to susceptible and resistant wheat cultivars. *Annals of the Entomological Society of America* **76**: 964-971.
- Allen, E.H. & Kuc, J.** 1968. a-Solanine and a-chaconine as fungitoxic compounds in extracts of Irish potato tubers. *Phytopathology* **58**: 776-781.
- Ames, B.N., Profet, M. and Gold, L.S.** 1990. Dietary pesticides (99.99% all natural). *Proceedings of the National Academy of Sciences* **87**: 7777-7781.
- Anderson, M. & Bromley, A.K.** 1987. Sensory system. In: Minks AK & Harrewijn P (eds), *Aphids Their Biology, Natural Enemies and Control*. 2A. Elsevier Science Publishers B.V. Amsterdam, pp. 153-162.
- Anonymous**, 1979. Solanine poisoning. *British Medical Journal* 1458-1459.
- Argandoña, V.H., Corcuera, L.J., Niemeyer, H.M. and Campbell, B.C.** 1983. Toxicity and feeding deterency of hydroxamic acids from gramineae in synthetic diets against the greenbug, *Schizaphis graminum*. *Entomologia Experimentalis et Applicata* **34**: 134-138.
- Argandoña, V.H., Luza, J.G., Niemeyer, H.M. and Corcuera, L.J.** 1980. Role of hydroxamic acids in the resistance of cereals to aphids. *Phytochemistry* 1665-1668.
- Argandoña, V.H., Niemeyer, H.M. and Corcuera, L.J.** 1981. Effect of content and distribution of hydroxamic acids in wheat on infestation by *Schizaphis graminum*. *Phytochemistry* **20**: 673-676.
- Argandoña, V.H., Zúñiga, G.E. and Corcuera, L.J.** 1987. Disrtibution of gramine and hydroxamic acids in barley and wheat leaves. *Phytochemistry* **26**: 1917-1918.
- Arneson, P.A. & Durbin, R.D.** 1968. Studies on the mode of action of tomatine as a fungitoxic agent. *Plant Physiology* **43**: 683-686.
- Auclair, J.L.** 1963. Aphid feeding and nutrition. *Annual Review of Entomology* **8**: 439-490.
- Auclair, J.L.** 1969. Nutrition of plant-sucking insects on chemically defined diets. *Entomologia Experimentalis et Applicata* **12**: 623-641.
- Augner, M.** 1995. Low nutritive quality as a plant defence: effects of herbivore-mediated interactions. *Evolutionary Ecology* **9**: 605-616.

- Ave, D.A., Gregory, P. and Tingey, W.M.** 1987. Aphid repellent sesquiterpenes in trichomes of *Solanum berthaultii* and *S. tuberosum*. *Entomologia Experimentalis et Applicata* **44**: 131-138.
- Bajaj, K.L., Singh, D. and Kaur, G.** 1989. Biochemical basis of relative field resistance of eggplant (*Solanum melongena*), to the shoot and fruit borer (*Leucinodes orbonalis* Guen.). *Vegetable Science* **16**: 145-149.
- Baldwin, I.T.** 1988. Mechanism of damage-induced alkaloid production in wild tobacco. *Journal of Chemical Ecology* **15**: 1661-1680.
- Baldwin, I.T.** 1988. Short-term damage-induced increases in tobacco alkaloids protects plants. *Oecologia* **75**: 367-370.
- Baldwin, I.T.** 1988. The alkaloidal responses of wild tobacco to real and stimulated herbivory. *Oecologia* **77**: 378-381.
- Barbosa, P. & Saunders, J.A.** 1985. Plant allelochemicals: linkages between herbivores and their natural enemies. In: Cooper-Driver GA, Swain TConn EE (eds), *Chemically Mediated Interactions Between Plants and Other Organisms*. 19. Plenum, New York, pp. 107-137.
- Bardner, R. & Fletcher, K.E.** 1974. Insect infestations and their effects on the growth and yield of field crops, a review. *Bulletin of Entomological Research* **64**: 141-160.
- Baron, R.L. & Guthrie, F.E.** 1960. A quantitative and qualitative study of sugars found in tobacco as affected by the green peach aphid, *Myzus persicae*, and its honeydew. *Annals of the Entomological Society of America* **53**: 220-228.
- Berenbaum, M.R.** 1986. Postingestive effects of phytochemicals on insects: on Paracelsus and Plant products. In: Miller JR & Miller TA (eds), *Insect/Plant Interactions*. Springer-Verlag, New York, pp. 121-154.
- Bergenstrahle, A., Tillberg, E. and Jonsson, L.** 1992. Regulation of glycoalkaloid accumulation in potato tuber discs. *Journal of Plant Physiology* **140**: 269-275.
- Bergenstrahle, A., Tillberg, E. and Jonsson, L.** 1992. Characterisation of UDP-glucose: solanidine glucosyltransferase and UDP-galactose:solanidine galactosyltransferase from potato tuber. *Plant Science* **84**: 35-44.
- Berlandier, F.A.** 1996. Alkaloid level in narrow-leaved lupin, *Lupinus angustifolius*, influences green peach aphid reproductive performance. *Entomologia Experimentalis et Applicata* **79**: 19-24.
- Bernays, E.A.** 1998. In: Lee JA, McNeill S, Rorison JH (eds), *Nitrogen as an Ecological Factor*. pp. 312-345.
- Bintcliffe, E.J.B., Clydesdale, A. and Draper, S.R.** 1982. Effects of genotype, site and season on the glycoalkaloid content of potato tubers. *Journal of the National Institute of the Agricultural Botany* **16**: 86-91.
- Birch, A.N.E., Griffiths, D.W., Hopkins, R.J., Smith, W.H.M. and McKinlay, R.G.** 1992. Glucosinolate responses of swede, kale, forage and oilseed rape to root damage by turnip root fly (*Delia floralis*) larvae. *Journal of the Science of Food and Agriculture* **60**: 1-9.
- Blackman, R.L.** 1988. Rearing and handling aphids. In: Minks AK & Harrewijn P (eds), *Aphids Their Biology, Natural Enemies and Control*. 2B. Elsevier Science Publishers B.V. Amsterdam, pp. 59-68.

- Boer de, G. & Hanson, F.E.** 1987. Feeding response of solanaceous allelochemicals by larvae of the tobacco hornworm, *Manduca sexta*. *Entomologia Experimentalis et Applicata* **45**: 123-131.
- Botha, C.E.J., Malcolm, S.B. and Evert, R.F.** 1977. An investigation of preferential feeding habit in four *Asclepiadaceae* by the aphid, *Aphis nerii* B. *Protoplasma* **92**: 1-19.
- Bradley, R.H.E.** 1952. Studies on the aphid transmission of a strain of henbane mosaic virus. *Annals of Applied Biology* **39**: 78-97.
- Bromley, A.K., Dunn, J.A. and Anderson, M.** 1979. Ultrastructure of the antennal sensilla of aphids. I. Coeloconic and placoid sensilla. *Cell and Tissue Research* **203**: 427-442.
- Bushway, A.A., Bushway, A.W., Beltea, P.R. and Bushway, R.J.** 1980. The proximate composition and glycoalkaloid content of three potato meals. *American Potato Journal* **57**: 167-171.
- Bushway, R.J., Savage, S.A. and Ferguson, B.S.** 1987. Inhibition of acetylcholinesterase by solanaceous glycoalkaloids and alkaloids. *American Potato Journal* **64**: 409-411.
- Caldwell, K.A., Grosjean, O.K., Henika, P.R. and Friedman, M.** 1991. Hepatic ornithine decarboxylase induction by potato glycoalkaloids in rats. *Food Chemistry and Toxicology* **29**: 531-535.
- Campbell, B.C. & Nes, W.D.** 1983. A reappraisal of sterol biosynthesis and metabolism in aphids. *Journal of Insect Physiology* **29**: 149-156.
- Caputo, R., Mangoni, L., Monaco, P. and Palumbo, G.** 1979. Triterpenes from the galls of *Pistacia palestina*. *Phytochemistry* **18**: 896-898.
- Chan, H.T.J. & Tam, S.Y.T.** 1985. Toxicity of a -tomatine to larvae of the Mediterranean fruit fly (Diptera: Tephritidae). *Journal of Economic Entomology* **78**: 305-307.
- Clayton, R.B.** 1964. The utilisation of sterols by insects. *Journal of Lipid Research* **5**: 3-19.
- Conner, H.W.** 1937. Effect of light on solanine synthesis in the potato tuber. *Plant Physiology* **12**: 79-98.
- Corcuera, L.J., Queirolo, C.B. and Argandoña, V.H.** 1985. Effects of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one on *Schizaphis graminum* Rondani (Insecta, Aphididae) feeding on artificial diets. *Experientia* **41**: 514-516.
- Coxon, D.T.** 1981. The glycoalkaloid content of potato berries. *Journal of the Science of Food and Agriculture* **32**: 412-414.
- Cronk, T.C., Kuhn, G.D. and McArdle, F.J.** 1974. The influence of stage of maturity, level of nitrogen fertilisation and storage on the concentration of solanine in tubers of three potato cultivars. *Bulletin of Environmental Contamination and Toxicology* **11**: 163-168.
- Dadd, R.H. & Mittler, T.E.** 1966. Permanent culture of an aphid on a totally synthetic diet. *Experientia* **12**: 832-833.
- Dahlman, D.L. & Hibbs, E.T.** 1967. Responses of *Empoasca fabae* (Cicadellidae: Homoptera) to tomatine, solanine, leptine I; solanidine, and demissidine. *Annals of the Entomological Society of America* **60**: 732-740.

- Dale, D.** 1988. Plant-mediated effects of soil mineral stresses on insects. In: Heinrichs EA (ed.), *Plant Stress-Insect Interactions*. John Wiley, New York, pp. 35-110.
- Dao, L. & Friedman, M.** 1996. Comparison of glycoalkaloid content of fresh and freeze-dried potato leaves determined by HPLC and colorimetry. *Journal of Agricultural and Food Chemistry* **44**: 2287-2291.
- Davidson, J.** 1923. Biological studies of *Aphis rumicis* Linn. The penetration of plant tissues and the source of the food supply of aphids. *Annals of Applied Biology* **10**: 35-54.
- Deahl, K.L., Cantelo, W.W., Sinden, S.L. and Sanford, L.L.** 1991. The effect of light intensity on Colorado potato beetle resistance and foliar glycoalkaloid concentration of four *Solanum chacoense* clones. *American Potato Journal* **68**: 659-666.
- Deahl, K.L., Young, R.J. and Sinden, S.L.** 1973. A study of the relationship of late blight resistance to glycoalkaloid content in fifteen potato clones. *American Potato Journal* **50**: 248-253.
- Dewar, A.M.** 1977. Assessment of methods for testing varietal resistance to aphids in cereals. *Annals of Applied Biology* **87**: 183-190.
- Diehl, S.G. & Chatters, R.M.** 1956. Studies on the mechanics of feeding of the spotted alfalfa aphid. *Journal of Economic Entomology* **49**: 589-591.
- Dilawari, V.K. & Atwal, A.S.** 1987. Effect of cruciferous glucosinolates on probing pattern and feed uptake by mustard aphid, *Lipaphis erysimi* (Kaltenbach). *Proceedings of the Indian Academy of Sciences (Anim.Sci.)* **96**: 695-703.
- Dillwith, J.W. & Berberet, R.C.** 1990. Lipids at the aphid-plant interface. In: Campbell RK & Eikenbary RD (eds), *Aphid-Plant Genotype Interactions*. Elsevier Science Publishers B.V. Amsterdam, pp. 207-223.
- Dixon, A.F.G.** 1971. The role of aphids in wood formation. I. The effect of the sycamore aphid, *Drepanosiphum platanoides* (Schr.) (Aphididae), on the growth of sycamore *Acer pseudoplatanus* (L.). *Journal of Applied Ecology* **8**: 165-179.
- Dixon, A.F.G.** 1971. The role of aphids in wood formation. II. The effect of the lime aphid, *Eucallipterus tiliae* L. (Aphididae), on the growth of lime *Tilia x vulgaris* Hayne. *Journal of Applied Ecology* **8**: 393-399.
- Dixon, A.F.G.** 1975. Aphids and translocation. In: Zimmerman MH & Milburn JA (eds), *Transport in plants I. Phloem transport*. Springer, Berlin, pp. 154-170.
- Dixon, A.F.G.** 1985. Host selection. *Aphid Ecology*. Blackie, Glasgow, pp. 6-22.
- Dixon, A.F.G.** 1987. Parthenogenetic reproduction and the rate of increase in aphids. In: Minks AK & Harrewijn P (eds), *Aphids Their Biology, Natural Enemies and Control*. 2A. Elsevier Science Publishers B.V. Amsterdam, pp. 269-287.
- Dixon, A.F.G. & Wratten, S.D.** 1971. Laboratory studies on aggregation, size and fecundity in the blackbean aphid, *Aphis fabae* Scop. *Bulletin of Entomological Research* **61**: 97-111.
- Dorschner, K.W., Johnson, R.C., Eikenbary, R.D. and Ryan, J.D.** 1986. Insect-plant interactions: green bugs (Homoptera: Aphididae) disrupt acclimation of winter wheat to drought stress. *Environmental Entomology* **15**: 118-121.
- Douglas, A.E.** 1988. On the source of sterols for the green peach aphid *Myzus persicae* on holidic diets. *Journal of Insect Physiology* **34**: 403-408.

- Douglas, A.E.** 1990. Nutritional interactions between *Myzus persicae* and its symbionts. In: Campbell RK & Eikenbary RD (eds), *Aphid-Plant Genotype Interactions*. Elsevier Science Publishers B.V. Amsterdam, pp. 319-327.
- Douglas, A.E.** 1996. Reproductive failure and the free amino acid pools in pea aphids (*Acyrtosiphon pisum*) lacking symbiotic bacteria. *Journal of Insect Physiology* **42**: 247-255.
- Dreyer, D.L., Jones, K.C. and Molyneux, R.J.** 1985. Feeding deterency of some pyrrolizidine, indolizidine, and quinolizidine alkaloids towards pea aphid (*Acyrtosiphon pisum*) and evidence for phloem transport of indolizidine alkaloid swainsonine. *Journal of Chemical Ecology* **11**: 1045-1051.
- Dreyer, D.L., Reese, J.C. and Jones, K.C.** 1981. Aphid feeding deterrents in sorghum. Bioassay, isolation and characterisation. *Journal of Chemical Ecology* **7**: 273-284.
- Dritschilo, W., Krummel, J., Nafus, D. and Pimentel, D.** 1979. Herbivorous insects colonising cyanogenic and acyanogenic *Trifolium repens*. *Heredity* **42**: 49-56.
- Edwards, E.J. & Cobb, A.H.** 1996. Improved high-performance liquid chromatographic method for the analysis of potato (*Solanum tuberosum*) glycoalkaloids. *Journal of Agricultural and Food Chemistry* **44**: 2705-2709.
- Ehrhardt, P.** 1968. Der Vitaminbedarf einer Siebrorensaugenden Aphide, *Neomyzus circumflexus* Buckt. (Homoptera Insecta). *Zeitschrift Vergleichende Physiologie* **60**: 416-426.
- Ehrhardt, P.** 1968. Nachweis einer durch symbiontische Mikroorganismen bewirkten Sterinsynthese in kunstlich ernahrte Aphiden (Homoptera, Rhynchotha, Insecta). *Experientia* **24**: 82-83.
- Eigenbrode, S.D. & Trumble, J.T.** 1994. Fruit-based tolerance to damage by armyworm (Lepidoptera: Noctuidae) in tomato. *Environmental Entomology* **23**: 937-942.
- van Emden, H.F.** 1966. Studies on the relations of insect and host plant. III. A comparison of the reproduction of *Brevicoryne brassicae* and *Myzus persicae* on Brussels sprout plants supplied with different rates of nitrogen and potassium. *Entomologia Experimentalis et Applicata* **9**: 444-460.
- van Emden, H.F.** 1969. Plant resistance to *Myzus persicae* induced by a plant regulator and measured by aphid relative growth rate. *Entomologia Experimentalis et Applicata* **12**: 125-131.
- van Emden, H.F.** 1971. Plant insect relationships in relation to control. *Pest Articles and News Summaries* **17**: 251-252.
- van Emden, H.F.** 1972. Aphids as phytochemists. In: Harborne JB (ed.), *Phytochemical Ecology*. Academic Press, London, New York, pp. 25-43.
- van Emden, H.F. & Bashford, M.A.** 1969. A comparison of the reproduction of *Brevicoryne brassicae* and *Myzus persicae* in relation to soluble nitrogen concentration and leaf age (leaf position) in the Brussels sprout plant. *Entomologia Experimentalis et Applicata* **12**: 351-364.
- van Emden, H.F., Eastop, V.F., Hughes, R.D. and Way, M.J.** 1969. The ecology of *Myzus persicae*. *Annual Review of Entomology* **14**: 197-270.
- Emrich, B.H. & Schmutterer, H.** 1990. Host-modulated biological characters of the lupin aphid *Macrosiphum albifrons* Essig (Homoptera: Aphididae). *Mededelingen*

van de Faculteit Landbouwwetenschappen, Rijksuniversiteit-Gent **55**: 463-470.

Eschrich, W. 1970. Biochemistry and fine structure of the phloem in relation to transport. *Annual Review of Plant Physiology* **21**: 193-214.

Feeny, P.P. 1976. Plant apparency and chemical defense. In: Wallace J & Mansell R (eds), *Biochemical Interactions Between Plants and Insects*. 10. Plenum, New York, pp. 1-40.

Fewell, A.M. & Roddick, J.G. 1993. Interactive antifungal activity of the glycoalkaloids α -solanine and α -chaconine. *Phytochemistry* **33**: 323-328.

Fewell, A.M., Roddick, J.G. and Weissenberg, M. 1994. Interaction between the glycoalkaloids solasonine and solamargine in relation to inhibition of fungal growth. *Phytochemistry* **37**: 1007-1011.

Forbes, J.C. & Watson, R.D. 1992. *Plants in Agriculture*. Cambridge University Press, Cambridge,

Forrest, J.M.S. & Coxon, D.T. 1980. The relationship between glycoalkaloids and resistance to the white potato cyst nematode *Globodera pallida* in potato clones derived from *Solanum vernei*. *Annals of Applied Biology* **94**: 265-268.

Forrest, J.M.S. & Knights, B.A. 1972. Presence of phytosterols in the food of the aphid *Myzus persicae*. *Journal of Insect Physiology* **18**: 723-728.

Frank, J.A., Wilson, J.M. and Webb, R.E. 1975. The relationship between glycoalkaloids and disease resistance in potatoes. *Phytopathology* **65**: 1045-1049.

Frazier, J.L. & Chyb, S. 1995. Use of feeding inhibitors in insect control. In: Chapman RF & Boer de G (eds), *Regulatory Mechanisms in Insect Feeding*. Chapman and Hall, New York, pp. 364-381.

Friedman, M. & Dao, L. 1992. Distribution of glycoalkaloids in potato plants and commercial potato products. *Journal of Agricultural and Food Chemistry* **40**: 419-423.

Friedman, M., McDonald, G. and Haddon, W.F. 1993. Kinetics of acid catalyzed hydrolysis of potato glycoalkaloids α -chaconine and α -solanine. *Journal of Agricultural and Food Chemistry* **41**: 1397-1406.

Friedman, M., Rayburn, J.R. and Bantle, J.A. 1991. Developmental toxicology of potato glycoalkaloids in the frog embryo teratogenesis assay-*Xenopus* (FETAX). *Food Chemistry and Toxicology* **28**: 537-547.

Friedman, M., Rayburn, J.R. and Bantle, J.A. 1992. Structural relationships and developmental toxicity of *Solanum* glycoalkaloids in the Frog Embryo Teratogenesis Assay-*Xenopus* (FETAX). *Journal of Agricultural and Food Chemistry* **40**: 1617-1624.

Frischknecht, P.M., Battig, M. and Baumann, T.W. 1987. Effect of drought and wounding stress on indole alkaloid formation in *Catharanthus roseus*. *Phytochemistry* **26**: 707-710.

Futuyma, D.J. 1976. Foodplant specialisation and environmental predictability in Lepidoptera. *American Naturalist* **110**: 285-292.

Ghidui, G.M., Carter, C. and Silcox, C.A. 1990. The effect of host plant on Colorado potato beetle (Coleoptera *Chrysomelidae*) susceptibility to pyrethroid insecticides. *Pesticide Science* **259**-270.

Gibson, R.W. 1971. Glandular hairs providing resistance to aphids in certain wild potato species. *Annals of Applied Biology* **68**: 113-119.

- Gibson, R.W. & Pickett, J.A.** 1983. Wild potato repels aphid by release of alarm pheromone. *Nature* **302**: 608-609.
- Gill, R.S. & Bakhetia, D.R.C.** 1985. Resistance of some *Brassica napus* and *B. campestris* strains to the mustard aphid. *Journal of Oilseeds Research* **2**: 227-239.
- Gosselin, B., Mondy, N.I. and Evans, W.D.** 1988. Effect of method of irrigation on the total glycoalkaloid and nitrate-nitrogen content of rosa potatoes. *American Potato Journal* **65**: 99-103.
- Gregory, P.** 1981. Glycoalkaloids of wild tuber bearing *Solanum* species. *Journal of Agricultural and Food Chemistry* **29**: 1212-1215.
- Gregory, P.** 1984. Glycoalkaloid composition of potatoes: diversity and biological implications. *American Potato Journal* **61**: 115-122.
- Griffiths, D.C., Greenway, A.R. and Lloyd, S.L.** 1978. The influence of repellent materials and aphid extracts on settling behaviour and larviposition of *Myzus persicae* (Sulzer) (Hemiptera:Aphididae). *Bulletin of Entomological Research* **65**: 613-619.
- Griffiths, G.W. & Beck, S.D.** 1977. In vivo sterol biosynthesis by pea aphid symbiotes as determined by digitonin and electron microscopic autoradiography. *Cell and Tissue Research* **176**: 179-190.
- Griffiths, G.W. & Beck, S.D.** 1977. Effect of dietary cholesterol on the pattern of osmium deposition in the symbiote-containing cells of the pea aphid. *Cell and Tissue Research* **176**: 191-203.
- Grime, J.P.** 1981. Plant strategies in shade. In: Smith H (ed.), *Plants and the Daylight Spectrum*. Academic Press, New York, pp. 159-186.
- Gull, D.D. & Isenberg, F.M.** 1960. Chlorophyll and solanine content and distribution in four varieties of potato tubers. *American Society of Horticultural Science* **75**: 545-556.
- Güntner, C., González, A., Dos Reis, R., González, G., Vázquez, A., Ferreira, F. and Moyna, P.** 1997. Effect of *Solanum* glycoalkaloids on potato aphid, *Macrosiphum euphorbiae*. *Journal of Chemical Ecology* **23**: 1651-1659.
- Guseva, A.R., Borikhina, M.G. and Paseshnichenko, V.A.** 1960. Utilization of acetate for the biosynthesis of chaconine and solanine in potato sprouts. *Biokhimiya* **25**: 282-284.
- Guseva, A.R. & Paseshnichenko, V.A.** 1958. A study of the biogenesis of potato glycoalkaloids by the method of labelled atoms. *Biokhimiya* **23**: 412-415.
- Hall, D.A. & Smith, C.A.** 1991. Will perlite beat peat? *Grower* 20-21.
- Hall, D.A. & Smith, C.A.** 1994. An evaluation of perlite-based substrates for ornamental pot plant production. *Acta Horticulturae* **361**: 486-490.
- Hammerschmidt, R.** 1993. The nature and generation of systemic signals induced by pathogens, arthropod herbivores, and wounds. *Advances in Plant Pathology*. 10. Academic Press, pp. 307-337.
- Han, S.R., Campbell, W.F. and Salunkhe, D.K.** 1989. Ultrastructural localization of solanidine in potato tubers. *Journal of Food Biochemistry* **13**: 377-389.
- Harborne, J.B.** 1977. In: Harborne JB (ed.), *Introduction to Ecological Biochemistry*. Academic Press, New York,
- Harborne, J.B.** 1993. The plant and its biochemical adaptation to the environment. *Introduction to Ecological Biochemistry*. Academic Press, London, pp. 1-35.

- Harborne, J.B.** 1993. Plant toxins and their effects on animals. In: Harborne JB (ed.), Introduction to Ecological Biochemistry. Academic Press, London, pp. 71-103.
- Harborne, J.B.** 1993. Insect feeding preferences. In: Harborne JB (ed.), Introduction to Ecological Biochemistry. Academic Press, London, pp. 128-161.
- Harrewijn, P.** 1970. Reproduction of the aphid *Myzus persicae* related to the mineral nutrition of potato plants. *Entomologia Experimentalis et Applicata* **13**: 307-319.
- Harrewijn, P.** 1976. Balance studies on the role of phosphate in host plant-aphid relationships. Proceedings of the International Congress on Soilless Culture IV. Las Palmas, pp. 339-346.
- Harrewijn, P.** 1978. The role of plant substances in polymorphism of the aphid *Myzus persicae*. *Entomologia Experimentalis et Applicata* **24**: 398-414.
- Harrewijn, P.** 1990. Resistance mechanisms of plant genotypes to various aphid species. In: Campbell RK & Eikenbary RD (eds), Aphid-Plant Genotype Interactions. Elsevier Science Publishers B.V. Amsterdam, pp. 117-130.
- Harrington, C.D.** 1945. Biological races of the pea aphid. *Journal of Economic Entomology* **38**: 12-22.
- Harrison, G.D. & Mitchell, B.K.** 1988. Host plant acceptance by geographic populations of the Colorado potato beetle, *Leptinotarsa decemlineata*. Role of solanaceous alkaloids as sensory deterrents. *Journal of Chemical Ecology* **14**: 777-788.
- Hartmann, T.** 1991. Alkaloids. In: Rosenthal GA & Berenbaum MR (eds), Herbivores: Their Interactions with Secondary Plant Metabolites. 1. The Chemical Participants. Academic Press, New York, pp. 79-121.
- Heftmann, E.** 1983. Biogenesis of steroids in *Solanaceae*. *Phytochemistry* **22**: 1843-1860.
- Heinrichs, E.A.** 1988. Global food production and plant stress. In: Heinrichs EA (ed.), Plant Stress-Insect Interactions. John Wiley, New York, pp. 1-33.
- Hellenas, K.E.** 1986. A simplified procedure for quantification of potato glycoalkaloids in tuber extracts by HPLC; Comparison with ELISA and a colorimetric method. *Journal of the Science of Food and Agriculture* **37**: 776-782.
- Hellenas, K.E. & Branzell, C.** 1995. Glycoalkaloids (solanine) in potatoes. *Var-Foda* **47**: 34-38.
- Hill, G.P.** 1962. Exudation from aphid stylets during the period from dormancy to bud break in *Tilia americana* (L.). *Journal of Experimental Botany* **13**: 144-151.
- Hilton, R.J.** 1951. Factors in relation to tuber quality in potatoes. II. Preliminary trials on bitterness in Netted Gem potatoes. *Scientific Agriculture* 61-70.
- Hlywka, J.J., Stephenson, G.R., Sears, M.K. and Yada, R.Y.** 1994. Effects of insect damage on glycoalkaloid content in potatoes (*Solanum tuberosum*). *Journal of Agricultural and Food Chemistry* **42**: 2545-2550.
- Houk, E.J.** 1987. Symbionts. In: Minks AK & Harrewijn P (eds), Aphids Their Biology, Natural Enemies and Control. 2A. Elsevier Science Publishers B.V. Amsterdam, pp. 123-129.
- Houk, E.J. & Griffiths, G.W.** 1980. Intracellular symbiotes of the Homoptera. *Annual Review of Entomology* **25**: 161-187.
- Houk, E.J., Griffiths, G.W. and Beck, S.D.** 1976. Lipid metabolism in the symbionts of the pea aphid, *Acyrtosiphon pisum*. *Comparative Biochemistry and*

Physiology **54b**: 427-431.

Hutchinson, A. & Hilton, R.J. 1955. The influence of certain cultural practices on solanine content and tuber yields in netted gem potatoes. *Canadian Journal of Agricultural Science* **35**: 485-491.

Jackson, M.B., Blackwell, P.S., Chrimes, J.R. and Sims, T.V. 1984. Poor aeration in NFT and a means for its improvement. *Journal of Horticultural Science* **59**: 439-448.

Jadhav, S.J. & Salunkhe, D.K. 1973. Enzymatic glucosylation of solanidine. *Journal of Food Science* **38**: 1099-1100.

Jadhav, S.J. & Salunkhe, D.K. 1975. Formation and control of chlorophyll and glycoalkaloids in tubers of *Solanum tuberosum* L. and evaluation of glycoalkaloid toxicity. *Advances in Food Research* **21**: 308-349.

Jadhav, S.J., Salunkhe, D.K., Wyse, R.E. and Dalvi, R.R. 1973. Solanum alkaloids: Biosynthesis and inhibition by chemicals. *Journal of Food Science* **38**: 453-455.

Jadhav, S.J., Sharma, R.P. and Salunkhe, D.K. 1981. Naturally occurring toxic alkaloids in foods. *CRC Critical Reviews in Toxicology* **9**: 21-67.

Jadhav, S.J., Wu, M.T. and Salunkhe, D.K. 1980. Glycoalkaloids of hollowheart and blackheart potatoes. *HortScience* **15**: 147-148.

Jaffe, M.J. & Telewski, F.W. 1984. Thigmomorphogenesis: callose and ethylene in the hardening of mechanically stressed plants. In: Timmermann BN, Steelink CLoewus FA (eds), *Phytochemical Adaptations to Stress*. 18. Plenum, New York, pp. 79-95.

Janick, J. 1974. Plant Science. In: Janick J, Schery RW, Woods FWRuttan VW (eds), *An Introduction to World Crops*. W.H. Freeman & Co, San Francisco,

Jansson, R.K. & Smilowitz, Z. 1986. Influence of nitrogen on population parameters of potato insects: abundance, population growth, and within-plant distribution of the Green peach aphid, *Myzus persicae* (Homoptera: Aphididae). *Environmental Entomology* **15**: 49-55.

Johnson, D., Heftmann, E. and Houghland, G.V.G. 1964. Biosynthesis of sterols in *Solanum tuberosum*. *Archives of Biochemistry and Biophysics* **104**: 102-118.

Johnston, K.A., Kershaw, W.J.S., and Pearce, R.S. 1989. Biochemical mechanisms of resistance of potato cultivars to slug attack. *BCPC*; **41** p.281.

Jonasson, T. & Olsson, K. 1994. The influence of glycoalkaloids, chlorogenic acid and sugars on the susceptibility of potato tubers to wireworm. *Potato Research* **37**: 205-216, 1994.

Jones, F.G.W. & Jones, M.G. 1984. Plant bugs-Hemiptera. *Pests of Field Crops*. Edward Arnold, pp. 36-72.

Kameko, K., Tanaka, M.W. and Mitsuhashi, H. 1976. Origin of nitrogen fertilisation in the biosynthesis of solanidine by *Veratrum grandiflorum*. *Phytochemistry* **15**: 1391

Kantack, E.J. & Dahms, R.G. 1957. A comparison of injury caused by the apple grain aphid and greenbug to small grains. *Journal of Economic Entomology* **50**: 156-158.

Keeler, R.F., Baker, D.C., Gaffield, W. 1991. Teratogenic *Solanum* species and the responsible teratogens. In: Keeler RF & Tu AT (eds), *Handbook of Natural Toxins*.

6. Marcel Dekker, Inc, New York, pp. 83-97.

Kennedy, G.G., Sorenson, C.E. and Fery, R.L. 1985. Mechanisms of resistance to Colorado potato beetle in tomato. *Research Bulletin, Massachusetts Agricultural Experimental Station* **704**: 107-116.

Kennedy, J.S. & Stroyan, H.L.G. 1959. Biology of aphids. *Annual Review of Entomology* **4**: 139-160.

Keukens, E.A.J., Jansen, L.A.M., de Boer, H. and de Kroon, 1996. Glycoalkaloids selectively permeabilize cholesterol containing biomembranes. *Biochimica et Biophysica Acta* **1279**: 243-250.

Khanna, P., Kumar, P. and Singhvi, S. 1998. Isolation and characterisation of solanine from in vitro tissue culture of *Solanum tuberosum* L and *Solanum dulcamara* L. *Indian Journal of Pharmaceutical Science* **50**: 37-38.

Kimmins, F.M. 1986. Ultrastructure of the stylet pathway of *Brevicoryne brassicae* in host plant tissue, *Brassicae oleracea*. *Entomologia Experimentalis et Applicata* **41**: 238-290.

Kimmins, F.M. & Tjallingii, W.F. 1985. Ultra structure of sieve element penetration by aphid stylets during electrical recording. *Entomologia Experimentalis et Applicata* **39**: 135-141.

Klashorst van de, G. & Tingey, W.M. 1979. Effect of seedling age, environment, temperature and foliar total glycoalkaloids on resistance of five *Solanum* genotypes to the potato leafhopper. *Environmental Entomology* **8**: 690-693.

Klingauf, F.A. 1987. Host plant finding and acceptance. In: Minks AK & Harrewijn P (eds), *Aphids Their Biology, Natural Enemies and Control*. 2A. Elsevier Science Publishers B.V. Amsterdam, pp. 209-223.

Klingauf, F.A. & Nocker-Wenzel, K. 1972. Einfluss von Aminosäuren auf das Wirtswahlverhalten von *Acyrtosiphon pisum* (Homoptera: Aphididae) unter besonderer Berücksichtigung ihres chemischen Aufbaus. *Entomologia Experimentalis et Applicata* **15**: 274-286.

Kloft, W. 1960. Wechselwirkungen zwischen pflanzensaugenden Insekten und den von ihnen besogenen Pflanzengewebe. Teil. I. *Zeitschrift für Angewandte Entomologie* **45**: 337-381.

Kogan, M. 1986. Natural chemicals in plant resistance to insects. *Iowa State Journal of Research* **60**: 501-527.

Kuhn, R. & Low, I. 1954. New alkaloid glycosides in the leaves of *Solanum chacoense*. *Angew Chemistry* **69**: 236-239.

Kuhn, R. & Low, I. 1955. Resistance factors against *Leptinotarsa decemlineata* (Say) isolated from the leaves of wild *Solanum* species. In: Sevag MG, Reid RD Reynolds OE (eds), *Origins of Resistance to Toxic Agents*. Academic Press, New York, pp. 122-132.

Laing, D. 1976. The soils of the country around Perth Arbroath and Dundee. Edinburgh, pp. 112-114.

Lambers, H. 1987. Growth, respiration, exudation and symbiotic associations: the fate of carbon translocated to roots. In: Gregory PJ, Lake JVRose DA (eds), *Root Development and Function*. Cambridge University Press, Cambridge, pp. 125-145.

Lampitt, L.H., Bushill, J.H., Rooke, H.S. and Jackson, E.M. 1943. Solanine glycoside of the potato. 1. It's distribution in the potato plant. *Journal of the Society*

of *Chemical Industry* **62**: 48-51.

Lampkin, N. 1990. Agriculture with a future. Organic Farming. Farming Press Books, pp. 1-10.

Leopold, A.C. 1975. In: Leopold AC & Kriedemann PE (eds), Plant Growth and Development. McGraw-Hill, USA,

Leszczynski, B., Wright, L.C. and Bakowski, T. 1989. Effect of secondary plant substances on winter wheat resistance to grain aphid. *Entomologia Experimentalis et Applicata* **52**: 135-139.

Levitt, J. 1980. Responses of Plants to Environmental Stresses. Academic Press, New York,

Liljegren, D.R. 1971. Glucosylation of solasodine by extracts from *Solanum laciniatum*. *Phytochemistry* **10**: 3061-3064.

Llewellyn, M., Rashid, R. and Leckstein, P. 1974. The ecological energetics of the willow aphid, *Tuberolachnus salignus* (Gmelin): Honeydew production. *Journal of Animal Ecology* **43**: 19-29.

Locci, R. & Kuc, J. 1967. Steroid alkaloids as compounds produced by potato tubers under stress. *Phytopathology* **57**: 1272-1273.

Lohar, M.K. 1989. Effect of an indole alkaloid, gramine, on the feeding behaviour of the aphids, *Schizaphis gramonum* (R.) and *Rhopalosiphum padi* (L.). *Pakistan Journal of Zoology* **21**: 261-266.

Long, B.J., Dunn, G.M., Bowman, J.S. and Routley, D.G. 1977. Relationship of hydroxamic acid content in corn and resistance to the corn leaf aphid. *Crop Science* **17**: 55-58.

Loper, G.M. 1968. Effects of aphid infestation on the coumestrol content of alfalfa varieties differing in aphid resistance. *Crop Science* **8**: 104-106.

Love, S.L., Herrman, T.J., Thompson-Johns, A. and Baker, T.P. 1994. Effect and interaction of crop management factors on the glycoalkaloid concentration of potato tubers. *Potato Research* **37**: 77-85.

Lowe, H.J.B. 1967. Interspecific differences in the biology of aphids (Homoptera:Aphididae) on leaves of *Vicia faba*. I. Feeding behaviour. *Entomologia Experimentalis et Applicata* **10**: 347-357.

MacGibbon, D.B. & Benzenberg, E.J. 1978. Location of glucosinolase in *Brevicoryne brassicae* and *Lipaphis erysimi* (Aphididae). *New Zealand Journal of Science* **21**: 389-392.

Mackenzie, J.D. & Gregory, P. 1979. Evaluation of a comprehensive method for total glycoalkaloid determination. *American Potato Journal* **56**: 27-33.

Mackenzie, J.D., Gregory, P. and Tingey, W.M. 1977. Foliar glycoalkaloid content of some wild potato species with differential resistance to green peach aphid and potato leafhopper. *American Potato Journal* **54**: 509

Malik, R.S. 1981. Morphological, anatomical and biochemical basis of aphid, *Lipaphis erysimi* Kalt., resistance in Cruciferous species. *Sveriges Utsadesforenings Tidskrift* **91**: 25-35.

Mansour, M.H., Dimetry, N.Z. and Rofael, I.S. 1982. The role of coumarin as secondary plant substance in the food specificity of the cow pea aphid *Aphis craccivora* Koch. *Zeitschrift fur Angewandte Entomologie* **93**: 151-157.

Marek, J. 1961. Uber das Einstich- und Saugverhalten der Zwiebellaus, *Myzus*

- ascalonicus* Doncaster. *Zeitschrift fur Pflanzenkrankheiten, Pflanzenpathologie und Pflanzenschutz* **68**: 155-165.
- McKee, R.K.** 1955. Host-parasite relationship in the dry rot disease of potatoes. *Annals of Applied Biology* **43**: 147-148.
- McLean, D.L. & Kinsey, M.G.** 1967. Probing behavior of the pea aphid *Acyrtosiphon pisum*. I. Definite correlation of electronically recorded wave forms with aphid probing activities. *Annals of the Entomological Society of America* **60**: 400-406.
- McLean, D.L. & Kinsey, M.G.** 1985. The precibarial valve and its role in the feeding behavior of the pea aphid, *Acyrtosiphon pisum*. *Bulletin of the Entomological Society of America* **30**: 26-31.
- McMillan, M. & Thompson, J.H.** 1979. An outbreak of suspected solanine poisoning in schoolboys: Examination of criteria of solanine poisoning. *Quarterly Journal of Medicine* **48**: 227-243.
- Meisner, J. & Mitchell, B.K.** 1984. Phagodeterrence induced by some secondary plant substances in adults of the flea beetle *Phyllotreta striolata*. *Zeitschrift fur Pflanzenkrankheiten, Pflanzenpathologie und Pflanzenschutz* **91**: 301-304.
- Mengel, K. & Kirkby, E.A.** 1987. Principles of Plant Nutrition. Potash Institute, Bern.
- Miles, P.W.** 1978. Redox reactions of hemipterous saliva in plant tissues. *Entomologia Experimentalis et Applicata* **24**: 334-339.
- Miles, P.W.** 1989. Specific responses and damage caused by Aphidoidea. In: Minks AK & Harrewijn P (eds), *Aphids Their Biology, Natural Enemies and Control*. 2C. Elsevier Science Publishers B.V. Amsterdam, pp. 23-47.
- Mitchell, B.K. & Harrison, G.D.** 1985. Effects of *Solanum* glycoalkaloids on chemosensilla in the Colorado potato beetle: A mechanism of feeding deterrence? *Journal of Chemical Ecology* **2**: 73-83.
- Mittler, T.E.** 1958. Studies on the feeding and nutrition of *Tuberolachnus salignus* (Gmelin) (Homoptera, Aphididae). III. The nitrogen economy. *Journal of Experimental Biology* **35**: 626-638.
- Mittler, T.E.** 1988. Applications of artificial feeding techniques for aphids. In: Minks AK & Harrewijn P (eds), *Aphids Their Biology, Natural Enemies and Control*. 2B. Elsevier Science Publishers B.V. Amsterdam, pp. 145-170.
- Mittler, T.E. & Dadd, R.H.** 1964. An improved method for feeding aphids on artificial diets. *Annals of the Entomological Society of America* **57**: 139-140.
- Mndolwa, D., Bishop, G., Corsini, D. and Pavek, J.** 1984. Resistance of potato clones to the green peach aphid and potato leafroll virus. *American Potato Journal* **61**: 713-722.
- Molyneux, R.J., Campbell, B.C. and Dreyer, D.L.** 1990. Honeydew analysis for detecting phloem transport of plant natural products. Implications for host-plant resistance to sap-sucking insects. *Journal of Chemical Ecology* **16**: 1899-1909.
- Monaco, P., Caputo, R., Palumbo, G. and Mangoni, L.** 1974. Triterpen components of galls on the leaves of *Pistacia terebinthus*, produced by *Pemphigus semilunarius*. *Phytochemistry* **13**: 1992-1993.
- Mondy, N.I., Leja, M. and Gosselin, B.** 1987. Changes in total phenolic, total glycoalkaloid and ascorbic acid content of potatoes as a result of bruising. *Journal of*

Food Science **52**: 631-633.

Mondy, N.I. & Munshi, C.B. 1990. Effect of nitrogen fertilization on glycoalkaloid and nitrate content of potatoes. *Journal of Agricultural and Food Chemistry* **38**: 565-567.

Mondy, N.I., Naylor, L.M. and Phillips, J.C. 1984. Total glycoalkaloid and mineral content of potatoes grown in soils amended with sewage sludge. *Journal of Agricultural and Food Chemistry* **32**: 1256-1260.

Montgomery, M.E. & Arn, H. 1974. Feeding response of *Aphis pomi*, *Myzus persicae*, and *Amphorophora agathonica* to phlorizin. *Journal of Insect Physiology* **20**: 413-421.

Moon, M.S. 1967. Phagostimulation of a monophagous aphid. *Oikos* **18**: 96-101.

Morgan, M.R.A. & Coxon, D.T. 1987. Tolerances: glycoalkaloids in potatoes. In: Watson DH (ed.), *Natural Toxicants in Food*. Ellis Horwood Series in food Science and Technology, pp. 221-230.

Morgenstern, F. 1998. Über den solanin Gehalt der Speise- und Futterkartoffeln und über den Einfluss der Bodenkultur auf die Bildung von Solanin in der Kartoffelpflanze. *LandwirtschVersStn* **65**: 301-309.

Morris, S.C. & Lee, T.H. 1984. The toxicity and teratogenicity of *Solanaceae* glycoalkaloids, particularly those of the potato (*Solanum tuberosum*): A review. *Food Technology in Australia* **36**: 118-124.

Morris, S.C. & Petermann, J.B. 1985. Genetic and environmental effects on levels of glycoalkaloids in cultivars of potato (*Solanum tuberosum* L.). *Food Chemistry* **18**: 271-282.

Munshi, C.B. & Mondy, N.I. 1988. Effect of soil applications of molybdenum on the biochemical composition of Katahdin potatoes: nitrate nitrogen and total glycoalkaloids. *Journal of Agricultural and Food Chemistry* **36**: 688-690.

Nair, P.M., Behere, A.G. and Ramaswamy, N.K. 1981. Glycoalkaloids of *Solanum tuberosum* Linn. *Journal of Scientific and Industrial Research* **40**: 529-535.

Nault, L.R. & Styer, W.E. 1972. Effects of sinigrin on host selection by aphids. *Entomologia Experimentalis et Applicata* **15**: 423-437.

Nielson, M.W. & Don, H. 1974. Probing behavior of biotypes of the spotted alfalfa aphid on resistant and susceptible alfalfa clones. *Entomologia Experimentalis et Applicata* **17**: 477-486.

Niemeyer, H.M. 1988. Hydroxamic acids (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-ones): defense chemicals in the Gramineae. *Phytochemistry* **27**: 3349-3358.

Niemeyer, H.M. 1990. The role of secondary plant compounds in aphid-host interactions. In: Campbell RK & Eikenbary RD (eds), *Aphid-Plant Genotype Interactions*. Elsevier Science Publishers B.V. Amsterdam, pp. 187-205.

Norris, D.M. 1988. Sensitivity of insect-damaged plants to environmental stresses. In: Heinrichs EA (ed.), *Plant Stress-Insect Interactions*. John Wiley, New York, pp. 341-361.

Nowacki, E.K., Jurzysta, M. and Gorski, P. 1975. Effect of availability of nitrogen on alkaloid synthesis in *Solanaceae*. *Bulletin de L'Academie Polonaise des Sciences Serie des sciences biologiques* **XXIII**: 219-225.

Nowacki, E.K., Jurzysta, M., Gorski, P., Nowacka, D. and Waller, G.R. 1976.

- Effect of nitrogen nutrition on alkaloid metabolism in plants. *Biochemie und Physiologie der Pflanzen* **169**: 231-240.
- Olsson, K.** 1986. The influence of genotype on the effects of impact damage on the accumulation of glycoalkaloids in potato tubers. *Potato Research* **29**: 1-12.
- Olsson, K.** 1989. Anatomical structure and chemical composition of potato tubers. In: Olsson K (ed.), Impact Damage, Gangrene and Dry Rot in Potato. Important Biochemical Factors in Screening for Resistance and Quality in Breeding Material. Swedish University of Agricultural Sciences, pp. 8-21.
- Olsson, K. & Jonasson, T.** 1995. Genotypic differences in susceptibility to wireworm attack in potato—mechanisms and implications for plant-breeding. *Plant Breeding* **114**: 66-69.
- Ortman, E.E. & Painter, R.H.** 1960. Quantitative measurements of damage by the greenbug *Toxoptera graminum*, to four wheat varieties. *Journal of Economic Entomology* **53**: 798-802.
- Osman, S.F., Herb, S.F., Fitzpatrick, T.J. and Sinden, S.L.** 1976. Commersonine, a new glycoalkaloid from two *Solanum* species. *Phytochemistry* **15**: 1065-1067.
- Osman, S.F. & Zacharius, R.M.** 1979. Biosynthesis of potato glycoalkaloids. *American Potato Journal* **56**: 475
- Osman, S.F., Zacharius, R.M. and Naglak, D.** 1980. Solanidine metabolism in potato tuber tissue slices and cell suspension cultures. *Phytochemistry* **19**: 2599-2601.
- Paseshnichenko, V.A.** 1957. Content of solanine and chaconine in the potato during the vegetation period. *Biokhimiya* **22**: 929-931.
- Percival, G.C.** 1993. Factors influencing accumulation of potato glycoalkaloids and their potential manipulation in tuber pathogen control (PhD Thesis). Scottish Agricultural College; p. 70
- Percival, G.C. & Dixon, G.R.** 1997. Glycoalkaloids. In: D'Mello J.P.F. (ed.), Handbook of Natural Toxins. CRC Press, pp. 19-35.
- Petitt, F.L. & Smilowitz, Z.** 1982. Green peach aphid feeding damage to potato in various plant growth stages. *Journal of Economic Entomology* **75**: 431-435.
- Petterson, D.S., Harris, D.J. and Allen, D.G.** 1991. Alkaloids. In D'Mello J.P.F., Duffus C.M. and Duffus J.H. (eds), Toxic Substances in Crop Plants. Royal Society of Chemistry, pp
- Pierzchalski, T.** 1957. Changes in the glycoalkaloid content in leaves of cultivated and wild potatoes and their hybrids during growth and their influence on the Colorado potato beetle (*Leptinotarsa decemlineata* Say). *Hodowla Roslin, Aklimatyzacja i Nasiennictwo* **1**: 415-430.
- Pollard, D.G.** 1973. Plant penetration by feeding aphids. *Bulletin of Entomological Research* **62**: 631-714.
- Prokoshev, S.M., Petrochenko, E.I. and Baranova, V.Z.** 1952. Glycoalkaloids of tuber bearing forms of *Solanum* in connection with their resistance to Colorado beetle. *Doklady Akademii Nauk (USSR)* **82**: 955-958.
- Puritch, G.S.** 1977. Distribution and phenolic composition of sapwood and heartwood in *Abies grandis* and effects of the balsam woolly aphid. *Canadian Journal of Forestry Research* **7**: 54-62.
- Puritch, G.S. & Nijholt, W.W.** 1974. Occurrence of juvabione-related compounds

- in grand fir and pacific silver fir infested by balsam woolly aphid. *Canadian Journal of Botany* **52**: 585-587.
- Qin Junde & Ke Lidao**, 1984. The influence of secondary plant substances on the growth and development of *Myzus persicae* of Beijing. *Entomologia Experimentalis et Applicata* **35**: 17-20.
- Rabbinge, R., Dress, E.M., Van der Graaf, M., Verberne, F.C.M. and Wesselo, A.** 1981. Damage effects of cereal aphids in wheat. *Netherlands Journal of Plant Pathology* **87**: 217-232.
- Raman, K.V., Iwanaga, M., Palacios, M. and Egusquiza, R.** 1981. Studies on identifying sources of resistance to potato tuberworm *Phthorimaea operculella* (Zeller). *American Potato Journal* **58**: 517
- Raman, K.V., Tingey, W.M. and Gregory, P.** 1979. Potato glycoalkaloids: effect on survival and feeding behavior of the potato Leafhopper. *Journal of Economic Entomology* **72**: 337-341.
- Ramaswamy, N.K., Behere, A.G. and Nair, P.M.** 1976. A novel pathway for the synthesis of solanidine in the isolated chloroplast from greening potatoes. *European Journal of Biochemistry* **67**: 275-282.
- Rayburn, J.R., Bantle, J.A. and Friedman, M.** 1994. Role of carbohydrate side chains of potato glycoalkaloids in the developmental toxicity. *Journal of Agricultural and Food Chemistry* **42**: 1511-1515.
- Reese, J.C.** 1979. Interactions of allelochemicals with nutrients in herbivore food. In: Rosenthal GA & Jansen DH (eds), *Herbivores: Their Interaction with Secondary Plant Metabolites*. Academic Press, New York, pp. 309-330.
- Reese, J.C. & Schmidt, D.J.** 1986. Physiological aspects of plant-insect interactions. *Iowa State Journal of Research* **60**: 545-567.
- Reeve, R.M., Hautala, E. and Weaver, M.L.** 1969. Anatomy and compositional variation within potatoes II. Phenolics, enzymes, and other minor components. *American Potato Journal* **37**: 374-386.
- Rhoades, D.F. & Cates, R.G.** 1976. Toward a general theory of plant antiherbivore chemistry. In: Wallace J & Mansell R (eds), *Biochemical Interactions Between Plants and Insects*. 10. Plenum, New York, pp. 168-213.
- Rhodes, M.J.C.** 1985. The physiological significance of plant phenolic compounds. In: Sumere van CF & Lea PJ (eds), *The Biochemistry of Plant Phenolics*. 25. Clarendon Press, Oxford, pp. 99-117.
- Robinson, T.** 1974. Metabolism and function of alkaloids in plants. *Science* **184**: 430-435.
- Roddick, J.G.** 1977. Subcellular localization of steroidal glycoalkaloids in vegetative organs of *Lycopersicon esculentum* and *Solanum tuberosum*. *Phytochemistry* **16**: 805-807.
- Roddick, J.G.** 1979. Complex formation between solanaceous steroidal glycoalkaloids and free sterols *in vitro*. *Phytochemistry* **18**: 1467-1470.
- Roddick, J.G.** 1982. Distribution of steroidal glycoalkaloids in reciprocal grafts of *Solanum tuberosum* L. and *Lycopersicon esculentum* Mill. *Experientia* **38**: 460-462.
- Roddick, J.G.** 1989. The acetylcholinesterase-inhibitory activity, steroidal glycoalkaloids and their aglycones. *Phytochemistry* **28**: 2631-2634.

- Roddick, J.G. & Rijnenberg, A.L.** 1986. Effect of steroidal glycoalkaloids of the potato on the permeability of liposome membranes. *Physiologia Plantarum* **68**: 436-440.
- Roddick, J.G. & Rijnenberg, A.L.** 1987. Synergistic interaction between the potato glycoalkaloids a-solanine and a-chaconine in relation to lysis of phospholipid/sterol liposomes. *Phytochemistry* **26**: 1325-1328.
- Roddick, J.G., Rijnenberg, A.L. and Osman, S.F.** 1988. Synergistic interaction between potato glycoalkaloids a-solanine and a-chaconine in relation to destabilisation of cell membranes: ecological implications. *Journal of Chemical Ecology* 889-902.
- Roddick, J.G., Rijnenberg, A.L. and Weissenberg, M.** 1990. Membrane disruptive properties of the steroidal glycoalkaloids solasonine and solamargine. *Phytochemistry* **31**: 1951-1954.
- Rothschild, M.J., Euw von, J. and Reichstein, T.** 1970. Cardiac glycosides in the Oleander aphid *Aphis nerii*. *Journal of Insect Physiology* **16**: 1141-1145.
- Ryan, C.A., Bishop, R., Pearce, G., Darvill, A.G., McNeil, M. and Albersheim, P.** 1981. A sycamore cell wall pectinase polysaccharide and a chemically related tomato polysaccharide possess similar proteinase inhibitor-inducing activities. *Plant Physiology* **68**: 616-618.
- Ryan, J.D., Morgham, A.T., Richardson, P.E., Johnson, R.C., Mort, A.J.,** 1990. Greenbugs and Wheat: A Model System for the Study of Phytotoxic Homoptera. In: Campbell RK & Eikenbary RD (eds), *Aphid-Plant Genotype Interactions*. Elsevier Science Publishers B.V. Amsterdam, pp. 171-186.
- Salas, M.L. & Corcuera, L.J.** 1991. Effect of environment on gramine content in barley leaves and susceptibility to the aphid *Schizaphis graminum*. *Phytochemistry* **30**: 3237-3240.
- Salas, M.L., Corcuera, L.J. and Argandoña, V.H.** 1990. Effect of potassium nitrate on gramine content and resistance of barley against the aphid *Schizaphis graminum*. *Phytochemistry* **29**: 3789-3791.
- Salunkhe, D.K. & Kadam, S.S.** 1991. Potato production processing and products. *CRC Press*
- Salunkhe, D.K., Wu, M.T. and Jadhav, S.J.** 1972. Effects of light and temperature on the formation of solanine in potato slices. *Journal of Food Science* **37**: 969-970.
- Sanford, L.L., Deahl, K.L., Sinden, S.L. and Ladd, T.L.J.** 1990. Foliar solanidine glycoside levels in *Solanum tuberosum* populations selected for potato leafhopper resistance. *American Potato Journal* **67**: 461-466.
- Sanford, L.L., Deahl, K.L., Sinden, S.L. and Ladd, T.L.J.** 1992. Glycoalkaloid contents in tubers from *Solanum tuberosum* populations selected for potato leafhopper resistance. *American Potato Journal* **69**: 693-703.
- Sanford, L.L., Domek, J.M., Cantelo, W.W., Kobayashi, R.S. and Sinden, S.L.** 1996. Mortality of potato leafhopper adults on synthetic diets containing seven glycoalkaloids synthesized in the foliage of various *Solanum* species. *American Potato Journal* **73**: 79-88.
- Schaper, P.** 1953. The resistance of *S. chacoense* to the Colorado potato beetle. *Zuchter* **23**: 115-121.
- Schepers, A.** 1989. Control of aphids; Chemical control. In: Minks AK & Harrewijn

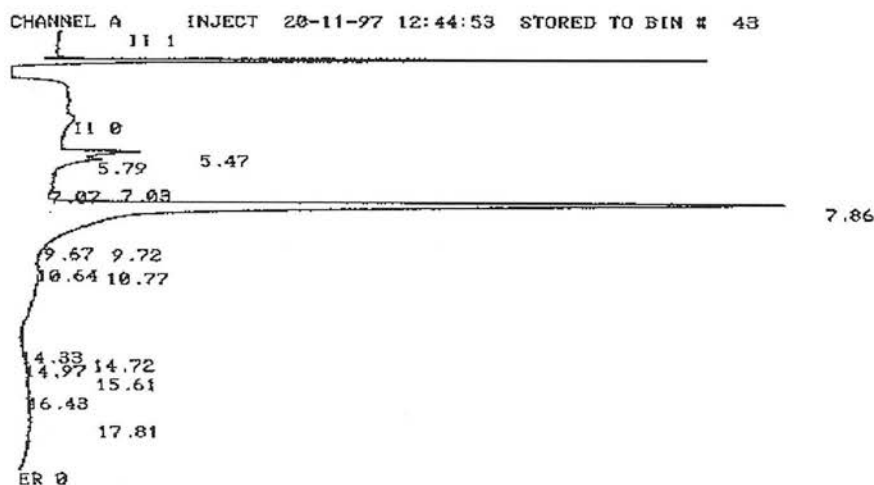
- P (eds), *Aphids Their Biology, Natural Enemies and Control*. 2C. Elsevier Science Publishers B.V. Amsterdam, pp. 89-122.
- Schneider, S.H. & Bach, W.** 1981. Interactions of food and climate: issues and policy considerations. In: Bach W, Pankrath JSchneider SH (eds), *Food-Climate Interactions*. Reidel, Dordrecht, Holland, pp. 1-19.
- Schoonhoven, L.M.** 1972. Secondary plant substances. In: Runeckles VC & Tso TC (eds), *Structural and Functional Aspects of Phytochemistry*. 5. pp. 197-224.
- Schoonhoven, L.M. & Derksen-Koppers, I.** 1976. Effects of some allelochemicals on food uptake and survival of a polyphagous aphid, *Myzus persicae*. *Entomologia Experimentalis et Applicata* **19**: 52-56.
- Schreiber, K.** 1957. Naturally occurring plant resistance factors against the Colorado potato beetle (*Leptinotarsa decemlineata*) and their possible mode of action. *Zuchter* **27**: 289-299.
- Schultz, J.C.** 1983. Impact of variable plant defensive chemistry on susceptibility of insects to natural enemies. In: Hedin PS (ed.), *Plant Resistance to Insects*. 208. American Chemical Society, Washington DC, pp. 37-54.
- Schwarze, P.** 1962. Methods for identification and determination of solanine in potato breeding material. *Zuchter* **32**: 155-160.
- Schwarze, P.** 1963. The glycoalkaloid content and the composition of the glycoalkaloid complex in progenies of the interspecific cross *Solanum tuberosum* x *S. chacoense*. *Zuchter* **33**: 275-281.
- Scott, T.K.** 1998. The Functions of hormones from the level of the cell to the whole plant. In: Scott TK (ed.), *Hormonal Regulation and Development*. 10. Springer-Verlag, Berlin-Heidelberg,
- Seigler, D.S. & Price, P.W.** 1976. Secondary compounds in plants: primary functions. *American Naturalist* **110**: 101-105.
- Simmonds, M.S.J., Blaney, W.M., Birch, A.N.E., and Stirton, C.H.** 1989. Aphid-legume interactions. Monographs in Systematic Botany from the Missouri Botanical Gardens 719 29.
- Sinden, S.L.** 1972. Effect of light and mechanical injury on the glycoalkaloid content of greening resistant potato cultivars. *American Potato Journal* **49**: 308
- Sinden, S.L., Goth, R.W. and O'Brien, J.** 1973. Effect of potato alkaloids on the growth of *Alternaria solani* and their possible role as resistance factors in potatoes. *Phytopathology* **63**: 303-307.
- Sinden, S.L., Sanford, L.L. and Osman, S.F.** 1980. Glycoalkaloids and resistance to the Colorado potato beetle in *Solanum chacoense* bitter. *American Potato Journal* **57**: 331-343.
- Sinden, S.L., Sanford, L.L. and Webb, R.E.** 1984. Genetic and environmental control of potato glycoalkaloids. *American Potato Journal* **61**: 141-156.
- Sinden, S.L. & Webb, R.E.** 1972. Effect of variety and location on the glycoalkaloid content of potatoes. *American Potato Journal* **49**: 334-338.
- Smith, B.D.** 1966. Effect of the plant alkaloid spartein on the distribution of the aphid *Acyrtosiphon spartii* (Koch.). *Nature* **212**: 213-214.
- Smith, C.A.** 1995; Possible feeding regimes for effect of N nutrition on the glycoalkaloid content of potatoes. *Personal Communication*.
- Speroni, J.J., Pell, E.J. and Weissberger, W.C.** 1981. Glycoalkaloid levels in

- potato tubers and leaves after intermittent plant exposure to ozone. *American Potato Journal* **58**: 407-414.
- Spiller, N.J., Kimmins, F.M. and Llewellyn, M.** 1985. Fine structure of aphid stylet pathways and its use in host plant resistance studies. *Entomologia Experimentalis et Applicata* **38**: 293-295.
- Srivastava, P.N.** 1987. Nutritional physiology. In: Minks AK & Harrewijn P (eds), *Aphids Their Biology, Natural Enemies and Control*. 2A. Elsevier Science Publishers B.V. Amsterdam, pp. 99-121.
- Steel, C.C. & Drysdail, R.B.** 1988. Electroligase from plant and fungal tissues and disruption of liposome membranes by α -tomatine. *Phytochemistry* **27**: 1025-1028.
- Stockdale, E.A., McKinlay, R.G. and Rees, R.M.** 1992. Soil nitrogen management and interaction with crop pests and diseases in organic farming. *Aspects of Applied Biology* **30**: 387-392.
- Sturckow, B. & Low, I.** 1961. The effects of some *Solanum* alkaloid glycosides on the potato beetle. *Entomologia Experimentalis et Applicata* **4**: 133-142.
- Szmidt, R.A.K., Hitchon, G.M. and Hall, D.A.** 1989. Sterilisation of perlite growing substrates. *Acta Horticulturae* **255**: 197-204.
- Smith, B.D.** 1966. Effect of the plant alkaloid spartein on the distribution of the aphid *Acyrtosiphon spartii* (Koch.). *Nature* **212**: 213-214.
- Tallamy, D.W. & Raupp, M.J.** 1991. In: Tallamy DW & Raupp MJ (eds), *Phytochemical Induction by Herbivores*. John Wiley and Sons, New York, pp. 431
- Thorsteinson, A.J.** 1960. Host selection in phytophagous insects. *Annual Review of Entomology* **5**: 193-218.
- Timmermann, B.N. & Steelink, C.** 1998. Introductory Chapter. In: Timmermann BN, Steelink C, Loewus FA (eds), *Phytochemical Adaptations to Stress*. 18. Plenum, New York, pp. 1-6.
- Tingey, W.M.** 1984. Glycoalkaloids as pest resistance factors. *American Potato Journal* **61**: 157-167.
- Tingey, W.M. & Laubengayer, J.E.** 1981. Defense against the green peach aphid and potato leafhopper by glandular trichomes of *Solanum berthaultii*. *Journal of Economic Entomology* **74**: 721-725.
- Tingey, W.M., Mackenzie, J.D. and Gregory, P.** 1978. Total foliar glycoalkaloids and resistance of wild potato species to *Empoasca fabae* (Harris). *American Potato Journal* **55**: 577-585.
- Tingey, W.M. & Sinden, S.L.** 1982. Glandular pubescence, Glycoalkaloid composition, and resistance to the green peach aphid, potato leafhopper, and potato flea beetle in *Solanum berthaultii*. *American Potato Journal* **59**: 95-106.
- Tjallingii, W.F.** 1985. Membrane potentials as an indication for plant cell penetration by aphid stylets. *Entomologia Experimentalis et Applicata* **38**: 187-193.
- Tjallingii, W.F.** 1995. Regulation of phloem sap feeding by aphids. In: Chapman RF & Boer de G (eds), *Regulatory Mechanisms in Insect Feeding*. Chapman & Hall, New York, pp. 190-209.
- Treshow, M.** 1970. *Environment and Plant Response*. McGraw-Hill, New York,
- Tschesche, R. & Hulpke, H.** 1967. Zur biosynthese von steroid derivaten in pflanzenreich. VIII. Biogenese von solanidin aus cholesterin. *Zeitschrift fur Naturforschung* **22**: 791

- Van Gelder, W.M.J., Vinke, J.H. and Scheffer, J.J.C.** 1988. Steroidal glycoalkaloids in tubers and leaves of *Solanum* species used in potato breeding. *Euphytica* **37**: 147-158.
- Waller, G.R. & Nowacki, E.K.** 1978. Environmental influences on alkaloid production. In: Waller GR & Nowacki EK (eds), *Alkaloid Biology and Metabolism in Plants*. Plenum Press, New York, London, pp. 85-119.
- Wearing, C.H.** 1968. Responses of aphids to pressure applied to liquid diet behind parafilm membrane. Longevity and larviposition of *Myzus persicae* (Sulz.) and *Brevicoryne brassicae* (L.) feeding on sucrose and sinigrin solutions. *New Zealand Journal of Science* **11**: 105-121.
- Weber, G., Oswald, S. and Zoeller, U.** 1986. Die Wirteseignung von Rapssorten unterschiedlichen Glucosinolatgehaltes für *Brevicoryne brassicae* (L.) und *Myzus persicae* (Sulzer) (Haemiptera: Aphididae). *Zeitschrift für Pflanzenkrankheiten, Pflanzenpathologie und Pflanzenschutz* **93**: 113-124.
- Wegorek, W. & Krzymanska, J.** 1968. Investigation on the biochemical causes of some lupine varieties resistant to pea aphid (*Acyrtosiphon pisum*, Harris). *Prace Naukowe-Institutu Ochrony Roslin* **10**: 7-30.
- Wegorek, W. & Krzymanska, J.** 1971. Further studies on the resistance of lupin to the pea aphid (*A.pisum*, Harris). *Prace Naukowe-Institutu Ochrony Roslin* **13**: 7-23.
- Weibull, J.** 1988. Free amino acids in the phloem sap from oats and barley resistant to *Ropalosiphum padi*. *Phytochemistry* **27**: 2069-2072.
- Weingartner, D.P., Shumaker, J.R. and Webb, R.E.** 1976. Failure of systemic insecticide-nematicides to affect total glycoalkaloid content of harvested potato tubers in northern Florida. *American Potato Journal* **53**: 247-252.
- Wensler, R.J.D.** 1962. Mode of host selection by an aphid. *Nature* **195**: 830-831.
- Wensler, R.J.D. & Filshie, B.K.** 1969. Gustatory sense organs in the food canal of aphids. *Journal of Morphology* **129**: 473-492.
- Wikteliu, S., Weibull, J., Pettersson, J.** 1990. Aphid host plant ecology: the bird cherry-oat aphid as a model. In: Campbell RK & Eikenbary RD (eds), *Aphid-Plant Genotype Interactions*. Elsevier Science Publishers B.V. Amsterdam, pp. 21-36.
- Willimot, S.C.** 1933. An investigation of solanine poisoning. *Analyst* **58**: 431-439.
- Wilson, J.M. & Frank, J.S.** 1975. The effect of systemic pesticides on total glycoalkaloid content of potato tubers at harvest. *American Potato Journal* **52**: 179-184.
- Wink, M.** 1983. Inhibition of seed germination by quinolizidine alkaloids. Aspects of allelopathy in *Lupinus albus* L. *Planta* **158**: 365-368.
- Wink, M.** 1988. Plant breeding: importance of plant secondary metabolites for protection against pathogens and herbivores. *Theoretical and Applied Genetics* **75**: 225-233.
- Wink, M., Hartman, T., Witte, L. and Rheinheimer, J.** 1982. Interrelationship between quinolizidine alkaloid producing legumes and infesting insects: exploitation of the alkaloid-containing phloem sap of *Cytisus scoparius* by the broom aphid *Aphis cytisorum*. *Zeitschrift für Naturforschung* **37**: 1081-1086.
- Wink, M. & Romer, P.** 1986. Acquired toxicity-the advantages of specializing on alkaloid-rich lupins to *Macrosiphon albifrons* (Aphididae). *Naturwissenschaften* **73**: 210-212.

- Witte, L., Ehmke, A. and Hartman, T. 1990. Interspecific flow of pyrrolizidine alkaloids. *Naturwissenschaften* **77**: 540-543.
- Wolf, M.J. & Duggar, B.M. 1946. Estimation and physiological role of solanine in the potato. *Journal of Agricultural Research* **71**: 1-32.
- Wood, F.A. & Young, D.A. 1974. Total glycoalkaloids in potatoes. *Canadian Agricultural Bulletin*
- Wool, D. & Hales, D.F. 1996. Previous infestation affects recolonisation of cotton by *Aphis gossypii* - induced resistance or plant-damage. *Phytoparasitica* **24**: 39-48.
- Wooldridge, A.W. & Harrison, F.P. 1968. Effects of soil fertility on abundance of green peach aphids on Maryland tobacco. *Journal of Economic Entomology* **61**: 387-391.
- Wratten, S.D. 1975. The nature and the effects of the aphids *Sitobion avenae* and *Metopolophium dirhodum*. *Annals of Applied Biology* **79**: 27-34.
- Wu, M.T. & Salunkhe, D.K. 1976. Changes in glycoalkaloid content following mechanical injuries to potato tubers. *Journal of the American Society of Horticultural Science* **101**: 329-331.
- Wu, M.T. & Salunkhe, D.K. 1978. Difference between light and wound induced biosynthesis of α -solanine and α -chaconine in potato tubers. *Biologia Plantarum* **20**: 149-151.
- Wyatt, I.J. & White, P.F. 1977. Simple estimation of intrinsic rates for aphids and tetranychid mites. *Journal of Applied Ecology* **14**: 757-766.
- Young, S. 1991. How plants fight back. *New Scientist* **1**: 41
- Zeigler, H. 1975. Nature of transported substances. In: Zimmerman MH & Milburn JA (eds), *Transport in Plants. I. Phloem Transport*. Springer, Berlin, pp. 59-100.
- Zitnak, A. 1961. The occurrence and distribution of free alkaloid solanidine in Netted Gem potatoes. *Canadian Journal of Biochemical physiology* **39**: 1257-1265.
- Zitnak, A. 1964. The significance of glycoalkaloids in potato plant. *Proceedings of the Canadian Society of Horticulture* **3**: 81-86.
- Zitnak, A. & Johnston, G.R. 1970. Glycoalkaloid content of B5141-6 potatoes. *American Potato Journal* **47**: 256-260.
- Zucker, W.V. 1982. How aphids choose leaves: the roles of phenolics in host selection by a galling aphid. *Ecology* **63**: 972-981.
- Zullo, M.A.T., Teixeira, J.P.F., Spoladore, D.S., Lourencao, A.L. and Aranha, C. 1984. Content of glycoalkaloids in *Solanum americanum* infested by *Aphis fabae solanella*. *Bragantia* **43**: 255-259.
- Zúñiga, G.E., Argandoña, V.H., Niemeyer, H.M. and Corcuera, L.J. 1983. Hydroxamic acid content in wild and cultivated Gramineae. *Phytochemistry* **22**: 2665-2668.
- Zúñiga, G.E. & Corcuera, L.J. 1986. Effect of gramine in the resistance of barley seedlings to the aphid *Rhopalosiphum padi*. *Entomologia Experimentalis et Applicata* **40**: 259-262.
- Zúñiga, G.E., Salgado, M.S. and Corcuera, L.J. 1985. Role of an indole alkaloid in the resistance of barley seedlings to aphids. *Phytochemistry* **24**: 945-947.
- Zúñiga, G.E., Varanda, E.M. and Corcuera, L.J. 1988. Effect of gramine on the feeding behaviour of the aphids *Schizaphis graminum* and *Rhopalosiphum padi*. *Entomologia Experimentalis et Applicata* **47**: 161-165.

Appendices

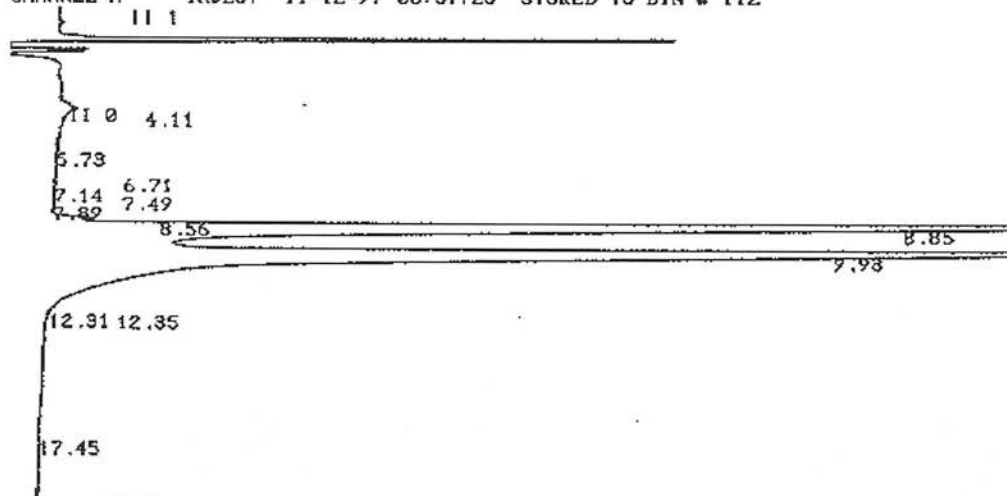


DATA SAVED TO BIN # 43

ALKALOIDS				20-11-97 12:44:53	CH= "A"	PS= 1.
FILE	S.	METHOD	Q.	RUN 33	INDEX 33	BIN 43
PEAK#	AREA	RT	AREA BC			
1	5.254	5.47	14996 02			
2	3.478	5.79	9925 03			
3	0.019	7.03	53 02			
4	0.053	7.07	156 03			
5	89.786	7.86	256254 08			
6	0.002	9.67	7 05			
7	0.004	9.72	10 05			
8	0.009	10.64	26 01			
9	0.164	10.77	469 01			
10	0.018	14.33	50 02			
11	0.222	14.72	685 02			
12	0.447	14.97	1275 02			
13	0.358	15.61	1023 02			
14	0.157	16.43	447 03			
15	0.028	17.81	80 01			
TOTAL	100.		295406			

Appendix 1. Chromatogram of α -solanine. Peak detected by UV absorbance at 202 nm: = α -solanine (retention time, R_t = 7.86 min) corresponding to standard of 62.5 ppm. Recorder setting at 0.002 absorbance units full scale (AUFS) output. Column: Phase Separations, model Spherisorb, packed with Shandon ODS2-hypersil 3 μ m; mobile phase acetonitrile/water: 34:66 (vol/vol) and 0.5ml/l ethanolamine adjusted to pH 4.55-4.56 with orthophosphoric acid (12% solution).

CHANNEL A INJECT 11-12-97 06:37:26 STORED TO BIN # 112



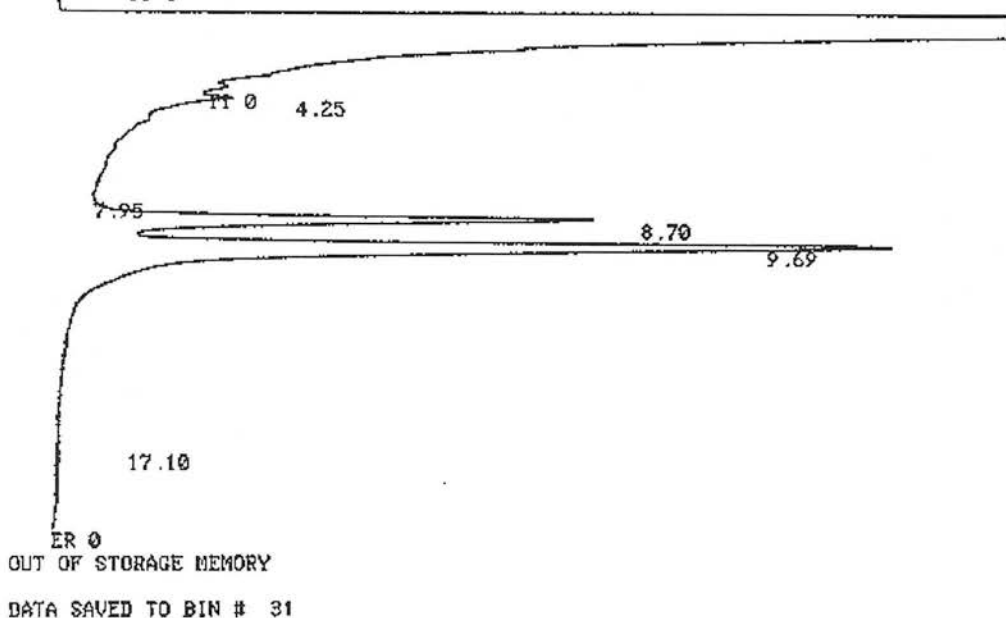
ER 0
OUT OF STORAGE MEMORY
DATA SAVED TO BIN # 112

ALKALOIDS				37:26	CH= "A"	PS= 1.
FILE 5.	METHOD 0.	RUN 482	INDEX 482	BIN 112		
PEAK#	AREA%	RT	AREA BC			
1	0.111	4.11	1159 01			
2	0.02	5.73	205 01			
3	0.023	6.71	237 02			
4	0.023	7.14	245 02			
5	0.014	7.49	151 02			
6	0.036	7.89	401 03			
7	0.607	8.56	6335 02			
8	43.609	8.85	455493 02			
9	55.526	9.93	579971 08			
10	0.001	12.31	14 05			
11	0.027	17.45	287 01			
TOTAL	100.		1044498			

Appendix 2. Chromatogram of α -solanine and α -chaconine. Peaks: = α -solanine (retention time, R_t = 8.85 min), α -chaconine (retention time, R_t = 9.93 min) corresponding to a standard of 125 ppm α -solanine and 125 ppm α -chaconine. All settings and adjustments as described in appendix 1.

CHANNEL A INJECT 29-11-97 05:34:15 STORED TO BIN # 31

11 1

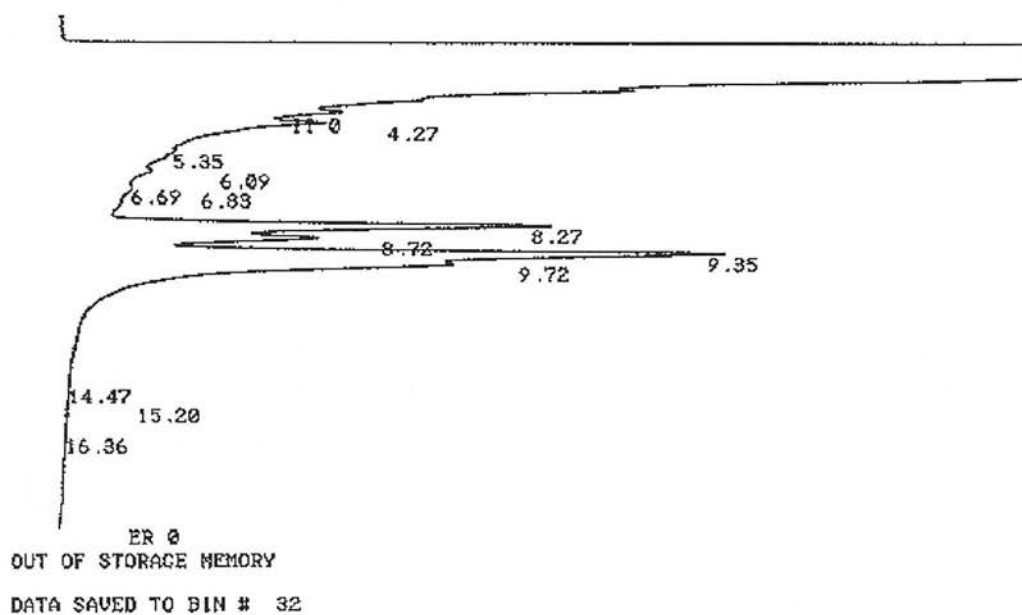


ALKALOIDS 29-11-97 05:34:15 CH= "A" PS= 1.

FILE 5. METHOD 0. RUN 148 INDEX 148 BIN 31

PEAK#	AREAX	RT	AREA BC
1	1.4	4.25	7732 01
2	0.015	7.95	85 02
3	30.699	8.7	169520 02
4	67.822	9.69	374515 03
5	0.064	17.1	354 01
TOTAL	100.		552206

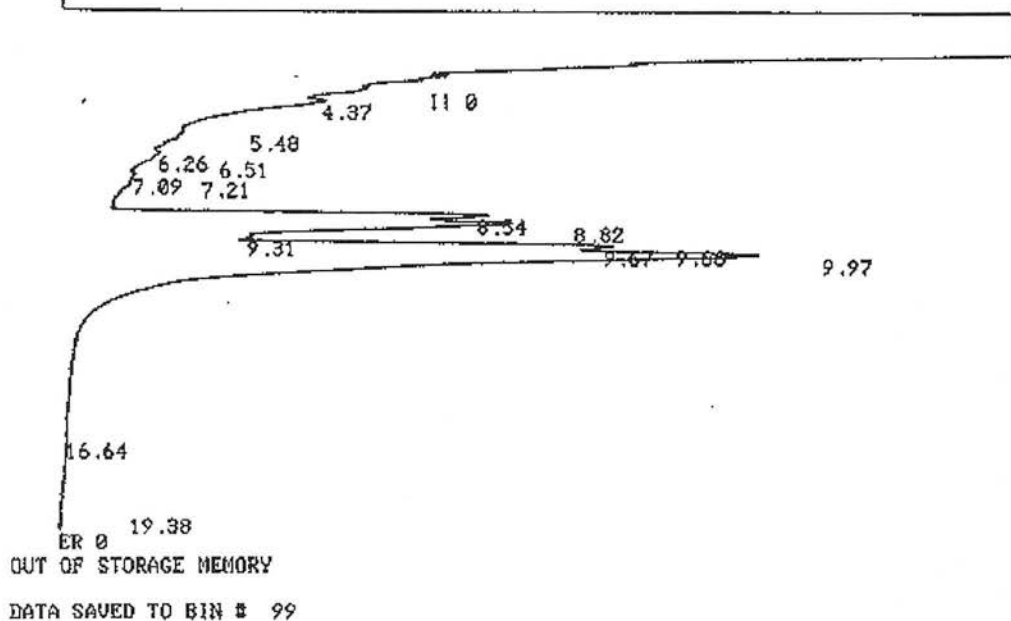
Appendix 3: Chromatogram of potato foliage extract (cv. King Edward). Peaks: A = α -solanine (retention time, R_t = 8.7 min) and B= α -chaconine (retention time, R_t = 9.69 min) corresponding to foliar tissue concentrations of 47.6mg/100g FWt and 85mg/100g FWt respectively. All settings and adjustments as described in appendix 1.



ALKALDIDS		29-11-97 05:56:41		CH= "A"	PS= 1.
FILE 5.	METHOD 0.	RUN 149	INDEX 149	BIN 32	
PEAK#	AREA X	RT	AREA BC		
1	2.405	4.27	14160 01		
2	0.045	5.35	265 01		
3	0.273	6.09	1610 01		
4	0.092	6.69	539 02		
5	0.136	6.83	802 03		
6	22.498	8.27	132464 02		
7	10.596	8.72	62387 02		
8	38.652	9.35	227577 02		
9	25.283	9.72	148867 03		
10	0.004	14.47	25 01		
11	0.01	15.2	58 01		
12	0.006	16.36	38 01		
TOTAL	100.		588792		

Appendix 4: Chromatogram of potato foliage extract (cv. Maris Piper). Peaks: A1 = α -solanine and A2 = possibly β -solanine (retention times, R_t = 8.27 and 8.72 min) and B1 = α -chaconine and B2 = possibly β -chaconine (retention times, R_t = 9.35 and 9.72 min) corresponding to foliar tissue concentrations of 44.9 mg/100g FWt, 21.1 mg/100g FWt, 62.3 mg/100g FWt and 40.8mg/100g FWt respectively. All settings and adjustments as in appendix 1.

CHANNEL A INJECT 30-11-97 07:26:46 STORED TO BIN # 99



ALKALOIDS		30-11-97 07:26:46		CH= "A"	PS= 1.
FILE 5.	METHOD 0.	RUN 215	INDEX 215	BIN 99	
PEAK#	AREA%	RT	AREA BC		
1	1.051	4.37	8775 01		
2	0.033	5.48	272 01		
3	0.222	6.26	1850 02		
4	0.076	6.51	635 03		
5	0.045	7.09	379 02		
6	0.005	7.21	38 03		
7	10.582	8.54	88328 02		
8	19.344	8.82	161461 02		
9	2.37	9.31	19782 02		
10	9.584	9.66	79998 02		
11	6.746	9.67	56308 02		
12	49.909	9.97	416578 03		
13	0.012	16.64	98 01		
14	0.021	19.38	172 01		
TOTAL	100.		834674		

Appendix 5: Chromatogram of potato foliage extract (cv. Maris Piper) indicating column blockage due to impurities in the mobile phase (twin split peaks). Reversing of the column and washing with methanol, acetonitrile, dichloromethane, acetonitrile, methanol and mobile phase each for 20 min led to removal of the impurities. New tests using α -solanine and α -chaconine standards were performed to check the system.

Published Papers

Fragoyiannis D.A., McKinlay R.G., D'Mello J.P.F., 1997. The effects of potato glycoalkaloids on the reproductive performance of the peach potato aphid. Proceedings of the Crop Protection and Food Quality meeting customer needs. Canterbury, 479-484.

Fragoyiannis D.A., McKinlay R.G., D'Mello J.P.F., 1998. Studies of the growth, development and reproductive performance of the aphid *Myzus persicae* on artificial diets containing potato glycoalkaloids. *Entomologia Experimentalis et Applicata*, 88: 59-66.

Studies of the growth, development and reproductive performance of the aphid *Myzus persicae* on artificial diets containing potato glycoalkaloids

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Abstract

Peach potato aphids *Myzus persicae* (Sulzer) (Homoptera: Aphididae) were reared on artificial diets containing the steroidal glycoalkaloids (GAs) α -solanine and α -chaconine in concentrations lower or similar to those observed in potato leaves. The adults proved to be susceptible to high concentrations (80–160 mg GA /100 ml of diet) showing reduced fecundity, diet uptake and increased mortality in comparison to controls. Ingestion of these artificial diets by nymphs delayed maturity and decreased the intrinsic rate of natural increase. GAs in low concentrations marginally stimulated the reproductive performance and diet acceptability of this aphid. The possibility of GAs exerting a defensive role in potato plants against aphids is discussed.

Introduction

Potato plants (*Solanum tuberosum* L. Solanaceae) contain natural steroidal toxins known as glycoalkaloids (GAs). The most abundant GAs found in potato are α -solanine and α -chaconine. Although GAs are considered to contribute to plant defence mechanisms possessing insecticidal properties (Tingey, 1984; Sanford et al., 1996), their role as potato plant allelochemicals in relation to the polyphagous peach-potato aphid *Myzus persicae* (Sulzer) (Homoptera: Aphididae), has been only partially investigated. In particular there is only one study examining the effects of α -solanine on *M. persicae* when offered in artificial diets in saturated solution. In food selection experiments using artificial diets, Schoonhoven & Derksen-Koppers (1976) showed that α -solanine did not enhance diet acceptability by *M. persicae* when incorporated to the basal diet, therefore this substance did not act as a feeding stimulant. Moreover, in a no choice situation, nymphs of *M. persicae* fed on an artificial diet containing α -solanine, showed normal survival after 2 and 4 days.

Little information exists in regard to the possible effects of these substances on the various life parameters of this aphid. The aim of this study was to investigate the lifespan effects of the two major potato GAs (α -solanine and α -chaconine) on the performance of *M. persicae* reared on artificial diets. The artificial diet method was used because in this way the possible effects of GAs on the aphid performance could be clearly observed, keeping constant other nutrients that could also affect aphid performance. The performance of the aphids was measured in terms of fecundity, longevity, diet acceptability, nymphal development and population growth rate.

Materials and methods

Biotic material

The aphids used in the experiments were a genetic clone of *M. persicae*, derived from one individual apterous female adult, which was collected on field grown oilseed rape on 8 June 1995, in Lothian Region

Scotland. The aphids were raised on Chinese cabbage leaves (*Brassica pekinensis* (Lour) Brassicaceae cv. Kasumi) in a controlled climate room at 25 °C, 50% r.h., and L 16:D 8 photoperiod.

Artificial diet

The GAs α -solanine and α -chaconine which were used in the experiments comprise 95% of the total potato foliar GAs (Jadhav et al., 1981). They were obtained (purity approx. 95%) from Sigma-Aldrich Co Ltd, Fancy Rd, Poole, Dorset, BH17 7NH England. The artificial diet prepared according to the recipe of Dadd & Mittler (1966) was used after preliminary investigations had indicated its suitability in terms of pH acceptability by the *M. persicae* clone. Two artificial diet stock solutions were prepared. In the first no GAs were added, while in the second the GAs were added at an initial concentration of 320 mg/100 ml to facilitate the artificial diet preparation by double dilution. The α -solanine to α -chaconine ratio (1:1.5) in this solution was similar to the ratio observed in potato leaves (Guseva et al., 1960). The different artificial diet treatments were derived by double diluting equal amounts of both solutions (GA-containing and non GA-containing) in volumetric flasks. Treatments were T₀, T₁₀, T₂₀, T₄₀, T₈₀, and T₁₆₀, representing control, 10, 20, 40, 80 and 160 mg of GA/100 ml of diet respectively. The GA concentration in the potato leaves ranges between 30–160 mg/100 g FW (Dao & Friedman, 1996). The initial pH after dilution was 4.6, but it was adjusted to 6.0 by adding a few drops from a 0.1 M KOH aqueous solution in each of the two solutions prepared. Although the original recipe suggested that the pH of an artificial diet should be 7.0, the modification of the pH proved to be essential, owing to the poor solubility of GAs in water solutions with pH higher than 5.6 (Mitchell & Harrison, 1985). In the GA containing stock solution, these substances were precipitated at a pH higher than 6.2. The osmolality values of the pure (0 mg of GA/100 ml of diet) and the GA-containing (160 mg of SGA/100 ml of diet) artificial diets were measured using a Wescor 5100C vapour pressure osmometer and were not found to be significantly different (822.25 mmol/kg, and 822.75 mmol/kg, respectively $t=0.47$ for 5df, $P>0.05$).

Procedures and assessment of aphid performance

Adult and nymph experiments were conducted in a controlled climate room at 25 °C, 50% r.h., and L 16:D 8 photoperiod.

Adult experiment. The adult aphid population used originated from nymphs born during a 12 h period on Chinese cabbage leaves. After their third ecdysis, the standardised 4th instar nymphs, left to starve for 12 h, were then transferred (using a moistened fine sable-hair brush) to glass cylinder cages for adaptation to the artificial diet substrate and caging conditions. The cages contained pure artificial diet (not containing SGAs) as a thin layer between two pieces of stretched Parafilm® membrane (Mittler & Dadd, 1964). The adults which completed the final ecdysis were transferred to the GA-containing artificial diet cages (4 replication-cages per artificial diet treatment, 5 apterous adult aphids per cage) to feed and reproduce. Individual fecundity (number of new born nymphs/apterous adult) and mortality (number of dead adult aphids/ cage) were measured with new born nymphs and dead adults counted and discarded every 24 h. The living adult aphids were transferred (every 48 h) to cages with fresh diet for a total of 34 days (entire aphid lifespan). Diet uptake for each diet-containing cage was calculated from the difference in the cage weight recorded before and after aphid feeding for the first 48 h of the experiment. Weight loss for each cage due to water evaporation was considered as equal for all artificial diet treatments.

Nymph experiment. The nymph population used was derived from female adults which were transferred from Chinese cabbage leaves to cages containing pure artificial diet where they remained for 6 h to reproduce. The standardised nymphs remained on the pure artificial diet for 12 h to gain weight and were then weighed using a Sartorius microbalance (model: Ultramicro) and transferred to the GA diet cages (4 replication-cages per artificial diet treatment, 15 nymphs per cage) to feed and develop in the same controlled climate room. The nymphs were reweighed and transferred to cages with fresh GA diet two more times before becoming adults. After their final ecdysis and before starting to reproduce, they were reweighed for the last time (as prereproductive adults) and five apterae were placed again on the diets to reproduce. From the weight measurements, the weight increase as well as the mean relative growth rate (MRGR) of

the living nymphs in each cage were calculated. The MRGR was calculated from the formula given by van Emden (1969):

$$\text{MRGR} = \frac{[\ln(\text{final weight}) - \ln(\text{initial weight})]}{[\text{growth period (days)}]}$$

The nymphs generally (with the exceptions of T₈₀ and T₁₆₀) took about 6.5 days before they themselves reproduced, and the next generation of nymphs they produced were counted, weighed and removed daily. In total the aphids fed on the diets 13 days on T₀–T₄₀ and 16 days on T₈₀. The Intrinsic Rate of Natural Increase (r_m) was then calculated for each cage of aphids according to the formula given by Wyatt & White (1977):

$$r_m = 0.74((\ln M_d)/d),$$

where d is the pre-reproductive time in days (from birth to first reproduction), and m_d is the number of progeny produced in the ensuing time period of length d . From a statistical viewpoint, the experiment was totally randomised with one treatment applied at six levels (T₀–T₁₆₀). Data were analysed separately at 4–6 different time points for each variable. The overall effect of the treatments was measured by a variance ratio test using MINITAB (vs. 11.1) and the differences between means measured by t-test.

Results

Adult experiment. Table 1 presents the fecundity data from the adult aphid experiment. There was a highly significant ($P < 0.001$) effect of SGA concentration on the mean daily offspring of the aphids due to the decreased mean number of new born nymphs observed in T₁₆₀ treatment. Aphids fed on T₈₀ treatment produced significantly fewer daily offspring compared to that of the control ($P < 0.05$), T₁₀ and T₂₀ ($P < 0.001$). Although the mean daily number of nymphs produced in low SGA concentrations (T₁₀, T₂₀) was significantly higher ($P < 0.05$) compared to that of the control, no significant ($P > 0.05$) differences were found between the total number of nymphs produced in control and T₁₀–T₂₀. In addition, no significant ($P > 0.05$) differences were found in the mean daily offspring produced between control and T₄₀, while the total number of nymphs produced in this treatment was significantly ($P < 0.01$) lower compared to that of the control.

The duration of the reproductive period appeared to be similar ($P > 0.05$) among control and GA treatments with the exception of the highly significantly

Table 1. Fecundity of *Myzus persicae* adults fed on artificial diets containing different concentrations of glycoalkaloids (GAs)

GA concentration mg/100ml	Mean daily offspring (nymphs/adult)	Mean duration of reproductive period (days)	Mean total progeny (nymphs/adult)
T ₀	2.09	21.8	45.57
T ₁₀	2.28	21.3	48.60
T ₂₀	2.30	19.3	44.40
T ₄₀	1.93	19.5	37.62
T ₈₀	1.85	19.0	35.22
T ₁₆₀	1.50	13.3	19.92
SED	0.09	1.4	2.41
P	***	***	***

SED: standard error of difference. *** $P < 0.001$.

($P < 0.001$) reduced reproductive period observed in the highest (T₁₆₀) GA concentration. The aphid mean total progeny was significantly ($P < 0.001$) affected by the GA concentration in the diet. In particular, highly significant ($P < 0.001$) differences were found between the total number of nymphs produced in control and T₈₀–T₁₆₀ treatments. Adult aphids fed on pure artificial diet produced 1.3 and 2.3 times more nymphs than those produced from aphids fed on T₈₀ and T₁₆₀, respectively. The total number of nymphs produced by aphids feeding on the two medium concentration (T₄₀, T₈₀) GA treatments appeared to be similar ($P > 0.05$), but significantly ($P < 0.001$) higher to the nymph number produced in T₁₆₀.

Figure 1 illustrates the reproductive performance of the aphids as this is expressed in the total number of nymphs born per adult in four day intervals for the entire reproductive period. Over this period, there was a significant ($P < 0.001$) effect of GA concentration on cumulative fecundity. This was largely due to the poorer fecundity observed on T₄₀, T₈₀ and T₁₆₀ treatments in comparison to control. In particular, during the first four days of feeding on the diets, the aphid reproductive performance was significantly lower in T₈₀ ($P < 0.01$) and T₁₆₀ ($P < 0.001$) compared to control, while no significant ($P > 0.05$) differences were observed in fecundity between control and T₄₀ treatments. In addition, highly significant ($P < 0.001$) differences were observed between medium (T₄₀–T₈₀) and high (T₁₆₀) treatments.

From the 8th to the 24th day of the experiment, significant ($P < 0.001$) differences in aphid reproductive performance were found between low (T₀, T₁₀, T₂₀), medium (T₄₀, T₈₀) and high (T₁₆₀) concentrations of

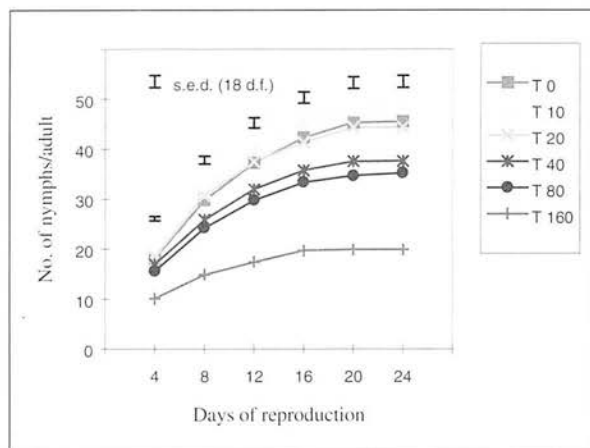


Figure 1. Mean cumulative fecundity of *Myzus persicae* adults fed on artificial diets containing different glycoalkaloid levels (T₀–T₁₆₀ mg/100 ml diet), at six different time intervals for the entire reproductive period (from 4 to 24 days).

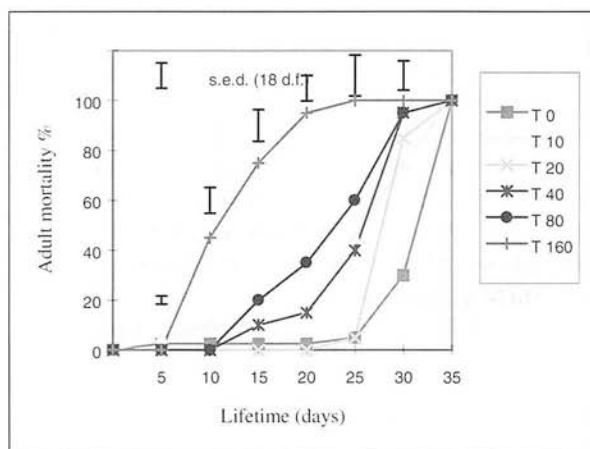


Figure 2. Mortality of *Myzus persicae* adults fed on artificial diets containing different concentrations of glycoalkaloids (T₀–T₁₆₀ mg/100 ml diet), at seven different time intervals across lifespan (from 5 to 35 days).

GAs. The highest fecundity was observed on T₁₀, but this was not significantly different ($P>0.05$) from the fecundity observed on control and T₂₀ treatments.

The effects of GAs on adult aphid longevity are shown in Figure 2. There were no significant ($P>0.05$) effects of GAs on longevity during the first five days of the experiment, while after ten days of feeding the effects of GAs were significant ($P<0.001$) in the highest (T₁₆₀) GA concentration treatment.

Most of the aphids did not settle on the diet containing the highest GA (T₁₆₀) treatment. On the 25th day of the experiment, the aphid mortality on T₁₀ was similar ($P>0.05$) to that observed on T₄₀ and T₈₀ and

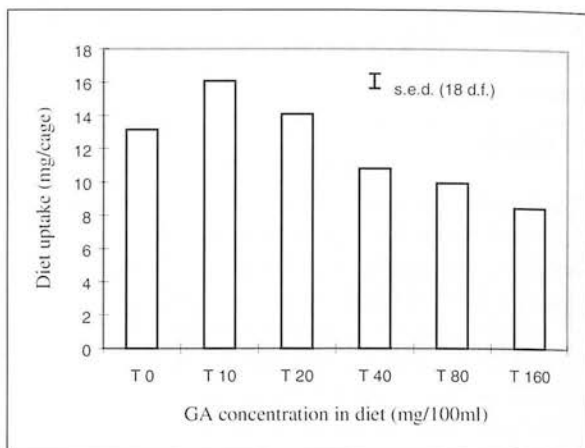


Figure 3. Diet uptake by *Myzus persicae* adults and offspring during 48 h of feeding on artificial diets containing different concentrations of glycoalkaloids (GAs).

significantly ($P<0.05$) higher than that observed on control and T₂₀.

The effects of GAs on the diet uptake of *M. persicae* for the first 48 hours of the experiment are presented in Figure 3. There was a significant ($P<0.001$) effect of GAs on diet uptake.

The uptake appeared to be highest in T₁₀ and lowest in T₁₆₀ treatments. In particular, the uptake observed in control was significantly higher in comparison to that observed in T₄₀ ($P<0.05$), T₈₀ ($P<0.01$) and T₁₆₀ ($P<0.001$). The diet uptake was significantly higher in T₁₀ than in control ($P<0.01$) and T₂₀ ($P<0.05$).

Nymph experiment. Figure 4 and Table 2 present data for nymph development on the artificial diets. Medium (T₄₀, T₈₀) and high (T₁₆₀) concentrations of GAs reduced the weight gain of nymphs (Figure 4) and led to a substantial (T₈₀) or infinite delay (T₁₆₀) of maturation.

In particular, the weight of nymphs fed for 60 h on T₄₀ was significantly ($P<0.05$) lower compared to control. This difference became highly significant ($P<0.001$) at the next weight measurement (120 h of feeding). There was a highly significant effect ($P<0.001$) of GAs on the weight gain of nymphs fed on T₈₀ and T₁₆₀ treatments. No adults were observed in T₈₀ and T₁₆₀ after 156 h of feeding, while the adults observed in T₄₀ were significantly ($P<0.001$) lighter than those observed in control.

Decreased weight gain of nymphs in the medium and high GA treatments led to a highly significant

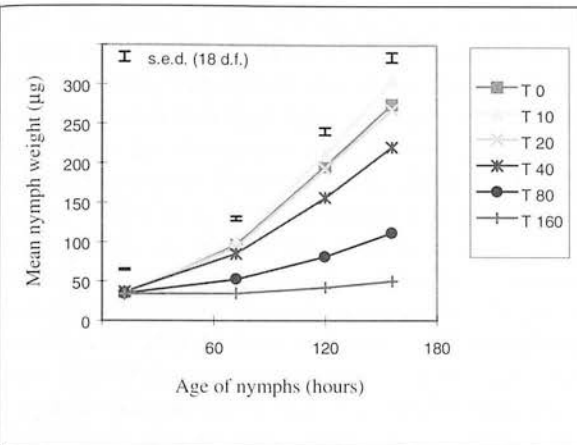


Figure 4. Weight increase of *Myzus persicae* nymphs reared on artificial diets containing different glycoalkaloid levels (T_0 – T_{160} mg/100 ml diet), at three different time intervals (after 60, 120 and 156 h of feeding).

Table 2. Life parameters (mean relative growth rate, nymph mortality and intrinsic rate of natural increase) of nymphs reared on artificial diets containing different concentrations of glycoalkaloids (GAs)

GA concentration mg/100 ml	Mean relative growth rate µg/µg day	Nymph mortality after 6.5 days (%)	Intrinsic rate of natural increase (r_m)
T_0	0.37	4.97	0.310
T_{10}	0.39	3.32	0.313
T_{20}	0.37	0.00	0.313
T_{40}	0.33	6.62	0.283
T_{80}	0.21	8.27	0.204
T_{160}	0.07	58.3	0.000
SED	0.01	4.04	0.008
P	***	***	***

SED: standard error of difference. *** $P < 0.001$.

($P < 0.001$) effect of GAs on their MRGR (Table 2). This was mainly due to poor development of nymphs observed in T_{80} and T_{160} treatments. Although pre-reproductive adults fed on T_{10} were significantly ($P < 0.05$) heavier compared to those fed on control, no significant ($P > 0.05$) differences were observed in the MRGRs between these two treatments. In addition, a highly significant ($P < 0.001$) difference in MRGR was observed between aphids fed on T_{40} and controls.

Additional data from the nymph experiment (Table 2) show that the intrinsic rate of natural increase, r_m , of *M. persicae* was similar ($P > 0.05$) among control and low (T_{10} , T_{20}) GA concentration

treatments. However the r_m values differed significantly ($P < 0.001$) between low (control, T_{10} , T_{20}) and medium (T_{40} , T_{80}) to high (T_{160}) concentrations of GAs. In particular, no offspring were produced from aphids fed on T_{160} treatment owing to increased nymph mortality and delayed nymph development, while there was a highly significant ($P < 0.001$) difference in the r_m values between T_{40} and T_{80} treatments.

No significant ($P > 0.05$) effects were observed on nymph mortality due to the GA presence in the diet in all treatments except in T_{160} ($P < 0.001$). All nymphs which fed on T_{20} treatment survived until maturity.

Discussion

The length of time required for an aphid to develop from birth to adult, the final adult weight, the reproductive rate and survival are dependent on food quality (Dixon, 1987). In our experiments, ingestion of an artificial diet containing 40 mg/100 ml or greater of potato GAs suppressed the reproductive performance (cumulative fecundity and intrinsic rate of increase), decreased the final weight of the prereproductive adults and delayed the development of nymphs to maturity.

Elevated levels of GAs in the diet reduced the number of nymphs born daily, as well as the number of nymphs produced during the entire aphid reproductive period which lasted 24 days (when the last born nymph was recorded). The reason for this elongated duration of measurements is that the reproductive potential of an aphid is formed in the very early stages of nymphal development and a possible effect of GAs on fecundity might not be detected by short term experimentation. Dixon (1987) suggested that embryos of a parthenogenetically reproducing aphid can have embryos developing within them. The GA presence in the diet reduced 1.09 and 1.51 times the intrinsic rate of natural increase on T_{40} and T_{80} treatments respectively compared to control, while no offspring was produced in the highest GA treatment. In this treatment the adult longevity was affected ten days after feeding on the diet, while the effects on nymphal longevity were observed earlier. This difference might be explained by the fact that in the adult experiment the aphids used had developed their nymphal stages on Chinese cabbage leaves, and it is therefore possible that the aphids had a higher tolerance compared to the nymphs used in the nymph experiment. Additional experimentation, involving electrical recording

of stylet penetration activities of *M. persicae* on artificial diets containing GAs, is essential to examine whether declined longevity and fecundity, observed in the higher GA treatments, were due to toxic effects of the GAs or feeding deterrence of the diets with subsequent starvation.

Marek (1961) suggested that *Myzus ascalonicus* (Donc) appeared to prefer to feed on buffer solutions of a pH-gradient ranging between 6.2 and 8.4 rather than on solutions of a higher or lower pH. In our experiments, *M. persicae* adapted well to the acidic (6.0) pH of the artificial diet, in contrast to the results of Schoonhoven & Derksen-Koppers (1976). They attributed a deterrent effect of their artificial diets on the feeding of *M. persicae* to the acidity caused by the incorporation of tannic acid in the diets. The difference between our results and those of Schoonhoven & Derksen-Koppers (1976) might be explained more by the repellent effect of tannic acid on aphid feeding than the acidity of the diets *per se*. An additional reason may be the possible differences between the biotypes of *M. persicae* used in our experiments and those that took place in the Netherlands. Furthermore, the fact that the aphids used in our experiments completed their final ecdysis in cages containing pure artificial diet (for adaptation purposes), might also have contributed to the differing results.

The same authors reported that α -solanine did not have a stimulatory effect when the artificial diet contained this substance in saturated concentration. They concluded that *M. persicae* nymphs showed normal survival after four days of feeding on the same diet. In our experiments, adult apterae reared on T₁₀ and T₂₀ diets produced higher daily offspring compared to control, indicating that there might be a short term stimulatory physiological role of GAs when they are presented to the aphids in low and not high (saturated) concentrations. This possibility is supported by the fact that diet uptake was highest in the lowest GA treatment (T₁₀). In addition the elevated mortality of nymphs and the delay/cancellation of maturity that were observed in our experiments suggest that the GAs negatively affect nymphal development when they are presented to the nymphs in elevated concentrations.

Güntner et al. (1997) found no strong lethal effects of α -solanine and α -chaconine when offered to the potato aphid *Macrosiphum euphorbiae* (Thomas) in concentrations up to 500 ppm, while α -chaconine stimulated its feeding at low concentrations. Therefore they concluded that the tolerance to GAs that *M. euphorbiae* was observed to exhibit, is an indication that

this aphid is well adapted to these substances. Our results, however, showed that *M. persicae* life parameters are negatively affected by diet concentrations similar to the medium and high GA concentrations (80–160 mg/100 FW equivalent to 800 and 1600 ppm, respectively) which can typically be detected in potato leaves (Dao & Friedman, 1996). A possible reason for this difference observed between the two species might be explained either by a higher tolerance of *M. euphorbiae* to potato GAs, or -most likely- by the fact that the GA concentrations used in the *M. euphorbiae* experiments (50–500 ppm) represent only the lower range of concentrations found in potato foliage. Comparative studies using artificial diets containing equal concentrations of GAs may be essential to indicate in more detail the differences in life parameters between these two aphid species.

Tingey & Sinden (1982) concluded that lack of significant correlation between total GA concentrations in accessions of *S. berthaultii*, (Hawkes) *S. berthaultii* \times *S. Tarijense*, (Hawkes) and peach potato aphid resistance levels did not conclusively rule out the involvement of GAs as a resistance factor to these pests. Griffiths et al. (1978) suggested that the plant tissues lying beneath the leaf surface but external to the phloem deserve more attention in studies on host plant selection. The route of aphid stylets to the phloem may be intercellular or intracellular, the latter regarded as more frequent (Tjallingii, 1985). *M. persicae* and in particular the first instar larvae were regarded as phloem as well as spongy mesophyll feeders (Lowe, 1967). Tjallingii (1985), using electrical penetration graphs, suggested that the short potential drops frequently produced during the stylet pathway to the phloem sieve elements – which are considered as the feeding sites of this aphid – correspond to intracellular stylet punctures through the epidermal or the mesophyllic cell membrane. It is therefore believed (Harrewijn, 1990; Niemeyer, 1990) that an aphid can locate plant resistance factors at the successive levels of the tissues encountered from the epidermis to the phloem, or can monitor the chemistry of the substrate and detect chemical gradients (van Emden, 1972); such an intake of sap samples for sensory purposes, however, has not been documented so far (Tjallingii, 1995). It is possible that during probing and/or feeding, *M. persicae* 'samples' GAs in the ingested sap, using the epipharyngeal organ which is in direct contact with fluid in the food canal and has the typical structure of a contact chemoreceptor (Wensler & Filshie, 1969). Although the actual time needed for the

stylets to penetrate the tissues from the epidermis to a sieve element is considered to be only a small proportion of the overall time required for sap feeding (Tjallingii, 1995), these insects require a minimum of 15 min to reach the phloem (Auclair, 1963). Therefore it seems that by feeding on phloem, aphids do not escape ingesting or coming into contact with a plant's defensive chemicals (Dixon, 1985), and in particular with GAs in concentrations present in the mesophyll. However the actual concentration of GAs in the mesophyll cells is not known. It follows therefore that the GA amounts imbibed by the aphid before the aphid stylet reaches the final feeding site, are not known. Furthermore, the fact that very low amounts of GAs stimulate food uptake, suggests that GAs may even have a stimulating effect on stylet penetration towards the phloem.

It has been suggested (Roddick, 1982) that although GA transport between root and shoot does not take place in potato, the transport of these substances is possible at the subcellular level, especially between the site of synthesis/glycosylation (probably particulate) and the site of accumulation (probably vacuolar). This is an indication that GAs are not present in the phloem, but further phloem sap analyses are required to document this hypothesis. It is appreciated that the studies reported in this paper are essentially toxicological and that the implication for field populations of aphids should also be addressed. Whether GAs act as resistance factors in the potato plant against *M. persicae*, or whether this aphid has the ability to avoid inducing their production *in situ*, remain unresolved questions.

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References

- Auclair, J. L., 1963. Aphid feeding and nutrition. *Annual Review of Entomology* 8: 439–490.
- Dadd, R. H. & T. E. Mittler, 1966. Permanent culture of an aphid on a totally synthetic diet. *Experientia* 12: 832–833.
- Dao, L. & M. Friedman, 1996. Comparison of glycoalkaloid content of fresh and freeze-dried potato leaves determined by HPLC and colorimetry. *Journal of Agricultural and Food Chemistry* 44: 2287–2291.
- Dixon, A. F. G., 1985. Host selection. In: *Aphid Ecology*. Blackie, Glasgow, pp. 6–22.
- Dixon, A. F. G., 1987. Parthenogenetic reproduction and the rate of increase in aphids. In: A. K. Minks & P. Harrewijn (eds), *Aphids, Their Biology, Natural Enemies and Control*. Vol. A. Elsevier Sc. Publ. B.V., Amsterdam, pp. 269–287.
- Emden, H. F. van, 1969. Plant resistance to *Myzus persicae* induced by a plant regulator and measured by aphid relative growth rate. *Entomologia Experimentalis et Applicata* 12: 125–131.
- Emden, H. F. van, 1972. Aphids as phytochemists. In: J. B. Harborne (ed.), *Phytochemical Ecology*. Academic Press, London, New York, pp. 25–43.
- Griffiths, D. C., A. R. Greenway & S. L. Lloyd, 1978. The influence of repellent materials and aphid extracts on settling behaviour and larviposition of *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). *Bulletin of Entomological Research* 65: 613–619.
- Güntner, C., A. González, R. Dos Reis, G. González, A. Vázquez, F. Ferreira & P. Moyna, 1997. Effect of *Solanum* glycoalkaloids on potato aphid, *Macrosiphum euphorbiae*. *Journal of Chemical Ecology* 23: 1651–1659.
- Guseva, A. R., M. G. Borikhina & V. A. Paseshnikchenko, 1960. Utilization of acetate for the biosynthesis of chaconine and solanine in potato sprouts. *Biokhimiya* 25: 282–284.
- Harrewijn, P., 1990. Resistance mechanisms of plant genotypes to various aphid species. In: R. K. Campbell & R. D. Eikenbary (eds), *Aphid-Plant Genotype Interactions*. Elsevier, Amsterdam, pp. 117–130.
- Jadhav, S. J., R. P. Sharma & D. K. Salunke, 1981. Naturally occurring toxic alkaloids in foods. *CRC Critical Reviews in Toxicology* 9: 21–67.
- Lowe, H. J. B., 1967. Interspecific differences in the biology of aphids (Homoptera: Aphididae) on leaves of *Vicia faba*. I. Feeding behaviour. *Entomologia Experimentalis et Applicata* 10: 347–357.
- Marek, J., 1961. Über das Einstich- und Saugverhalten der Zwiebellaus, *Myzus ascalonicus* Doncaster. *Zeitschrift für Pflanzenkrankheiten, Pflanzenpathologie und Pflanzenschutz* 68: 155–165.
- Mitchell, B. K. & G. D. Harrison, 1985. Effects of *Solanum* glycoalkaloids on chemosensilla in the Colorado potato beetle: A mechanism of feeding deterrence? *Journal of Chemical Ecology* 2: 73–83.
- Mittler, T. E. & R. H. Dadd, 1964. An improved method for feeding aphids on artificial diets. *Annals of the Entomological Society of America* 57: 139–140.
- Niemeyer, H. M., 1990. The role of secondary plant compounds in aphid-host interactions. In: R. K. Campbell & R. D. Eikenbary (eds), *Aphid-Plant Genotype Interactions*. Elsevier, Amsterdam, pp. 187–205.
- Roddick, J. G., 1982. Distribution of steroidal glycoalkaloids in reciprocal grafts of *Solanum tuberosum* L. and *Lycopersicon esculentum* Mill. *Experientia* 38: 460–462.
- Sanford, L. L., J. M. Domek, W. W. Cantelo, R. S. Kobayashi & S. L. Sinden, 1996. Mortality of potato leafhopper adults on synthetic diets containing seven glycoalkaloids synthesized in the foliage of various *Solanum* species. *American Potato Journal* 73: 79–88.
- Schoonhoven, L. M. & I. Derksen-Koppers, 1976. Effects of some allelochemicals on food uptake and survival of a polyphagous

- aphid, *Myzus persicae*. *Entomologia Experimentalis et Applicata* 19: 52–56.
- Tingey, W. M., 1984. Glycoalkaloids as pest resistance factors. *American Potato Journal* 61: 157–167.
- Tingey, W. M. & S. L. Sinden, 1982. Glandular pubescence, glycoalkaloid composition, and resistance to the green peach aphid, potato leafhopper, and potato flea beetle in *Solanum berthaultii*. *American Potato Journal* 59: 95–106.
- Tjallingii, W. F., 1985. Membrane potentials as an indication for plant cell penetration by aphid stylets. *Entomologia Experimentalis et Applicata* 38: 187–193.
- Tjallingii, W. F., 1995. Regulation of phloem sap feeding by aphids. In: R. F. Chapman & G. de Boer (eds), *Regulatory Mechanisms in Insect Feeding*. Chapman & Hall, New York, pp. 190–209.
- Wensler, R. J. & B. K. Filshie, 1969. Gustatory sense organs in the food canal of aphids. *Journal of Morphology* 129: 473–492.
- Wyatt, I. J. & P. F. White, 1977. Simple estimation of intrinsic rates for aphids and tetranychid mites. *Journal of Applied Ecology* 14: 757–766.